JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., NOVEMBER 15, 1938 No. 10

SAMPLING ERROR IN TIMBER SURVEYS[1] [2]

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INTRODUCTION

In cruising timber, the mean volume of any given area is usually estimated from the mean volume per unit area of a small percentage of the timberland taken as a sample. This estimated mean volume will inevitably differ to some extent from the true mean volume. With the elimination of such factors as bias in measurement of trees and of areas or use of inappropriate volume tables, the probable range of difference between true mean volume and estimated mean volume is dependent upon sampling error alone. If the plots or sampling units making up the sample meet the conditions of independent and random selection, the sample itself contains the information needed for estimating sampling error.

While it is of interest to know with what precision the estimated mean has been found, it is vitally more important from an economic standpoint to be able to predetermine what shall be a sufficient sample for an assumed allowable range of error. If the sampling error is not already known, it is necessary to take a preliminary sample of the area to gain this information. From this, it is possible to establish the intensity of the cruise that will produce results within the assumed allowable range of error of the mean. It is then necessary only to provide for occasional supplementary checks, as the cruise progresses, for adequacy of the work done.

In regular cruising practice a systematic arrangement of plots is used in which the sample is made up of contiguous plots forming equidistant strips, or of plots taken at regular intervals along equidistant lines. In most timber surveys, cover type and topographic maps are made in conjunction with the cruise, and for this purpose it is desirable that cruise lines be spaced equidistant in order that all parts of the area may be mapped to a satisfactory standard. As stand and type variation are generally greatest at right angles to contour lines, the cruise lines are usually run in that direction, and plots are spaced closer along lines than between lines. By this procedure type and contour lines are more readily located and mapped, and the more intensive sampling in the direction of greatest variation improves the accuracy of volume estimates.

This arbitrary spacing of plots presents difficulties, should a test of adequacy of sampling be attempted by treatment as a random sample in statistical analysis, since it violates the basic requirement that each possible plot in an area have an equal and independent

[1] Received for publication April 26, 1938; issued December 1938.
[2] Appreciation is extended to the Works Progress Administration for assistance rendered by statistical workers employed on Project No. 365-03-3-35.
[3] Maintained at Berkeley, Calif., in cooperation with the University of California.

chance of selection in sampling. Experiments in field crops have shown time and again that there is systematic variation in yield from one part of an area to another. Adjacent or neighboring plots tend on the average to be more alike than plots farther apart. Without previous knowledge of an area, it should be assumed that a population of plots is more or less heterogeneous, regardless of appearance of uniformity. The only method of assuring that the elements contributing to heterogeneity are represented in the sample in about their true proportion is to select at random the plots or parts of a sample contributing the estimate of sampling error. The plots indicated for cruising may, for example, be selected by drawing from thoroughly mixed numbers designating each possible plot location. After each draw, the numbered slip is returned and the numbers mixed before another draw. Repeats of plot numbers are rejected, as the objective is a fixed percentage of total area in the sample with each plot selected contributing the same amount of information per unit of area. An improvement over this method, assuring the same freedom of selection, is the use of Tippett's random sampling numbers (*11*).[4] The locating of such plots on the ground may be less convenient than with systematically spaced plots and may also make necessary the running of additional line for mapping. This is the sacrifice necessary for assurance of a valid estimate of error.

The fact that the sampling of timber stands, except for technique in collection of data, is essentially the same as any other problem in sampling in which soil heterogeneity is likely to be present, has not been fully recognized. Apparently advantage has not been taken of methods of testing for heterogeneity and, if indicated, of eliminating its effect on estimates of sampling error—methods first proposed and described by Fisher and Mackenzie (*3*) in 1923. Areas considered as of the same timber type, condition class, age class, and site quality have been treated as homogeneous populations. Various arbitrary plot spacings have been used. Mudgett and Gevorkiantz (*6*) in estimating the reliability of forest surveys in Wisconsin used plots at one-eighth mile intervals along parallel lines 1, 2, or 3 miles apart. Within the break-downs made in a type the errors of random sampling were based on total variation between systematically arranged plots. In the bottom-land hardwood forest survey, Schumacher and Bull (*9*) based their estimate of error on plots at one-eighth mile intervals along lines 3 miles apart. The plots were grouped according to forest condition, i. e., virgin, cut-over, second growth, etc., and the weighted mean of the standard deviations so obtained was used in estimating sampling error. In New England, Goodspeed (*4*) compared line-plot survey, using plots at 165-foot intervals along lines 330 feet apart, with continuous-strip survey along the same lines, taking each 165-foot length of strip as an independent observation. Since the strips and plots overlapped 44.5 percent, variation in sampling was actually confined to 55.5 percent of each sample. From statistical analysis of the data he concluded that the two methods, when applied with equal precision, gave essentially similar and equally reliable results. Preston (*7*) recommended statistical analysis of systematic line-plot cruises and suggested that timber types be mapped separately and prior to sampling. He pointed out the futility of adhering to a 5- or 10-percent, or any other fixed, preconceived intensity of cruise.

[4] Italic numbers in parentheses refer to Literature Cited, p. 735.

In Canada, Wright (*12*) used plots at 10-chain intervals along lines 10 chains apart. In strip survey his strips were run at half-mile intervals, and the tally kept separately by 2-chain segments. Seven estimates were made by taking the first 6 chains in each half mile of strip as a plot, the next 6 chains for a plot in the second sample, and so on to the seventh sample which was made up of plots 4 chains in length. The average of these seven sample estimates of variation was applied to the mean of all plots in calculating sampling error. Wright stated that the main requirement in the use of statistical methods in examining the accuracy of an estimate was to have a reliable figure for standard deviation. Robertson (*8*) favored small plots evenly distributed over the area as against a few hand-picked large plots, with analysis of data from time to time during the survey to insure the taking of sufficient plots to give an accuracy within the required limits for any given factor. The line-plot arrangement he found to be more accurate than the strip. Since in strip survey the plots are contiguous in one direction, he suggested that they represented conditions in that direction beyond their due proportion. Candy (*1*) stated that any method of survey for which it is possible to calculate the accuracy of the estimate obtained, is very much superior to methods in which the accuracy of estimate is doubtful and not at all calculable. He used both systematic strip and line plot, and concluded that only with line plots could adequacy of sampling be determined.

In the present study the true mean volume and the true variation between plots are known for an area of 5,760 acres. The distribution of plot volumes is known to approach that of the normal frequency distribution. Thus all of the essential statistical information regarding the population is at hand. The expected range of sample means from the true mean is calculated, taking into account the effect of such factors as size, shape, arrangement of plots, and intensity of sampling. From samples taken according to the specifications set up, the observed range of means from the true is compared with the expected range. Checks of theoretical with actual efficiency of sampling methods are readily made, since the timbered area studied is part of the Blacks Mountain experimental forest, in which the total timbered area of 9,078 acres has been completely inventoried and plot locations, cover types, and topography mapped. The usual assumption that systematically spaced plots do fulfill the requirements of independent and random selection is tested, both for strip and line-plot arrangements. Efficiency of different methods of cruising is determined on the basis of relative intensity of sampling required for a given accuracy of estimate. For cruises giving valid estimates of sampling error, the precision of sample estimates of population variance is given.

MATERIAL AND METHODS

The 5,760 acres of virgin timber on which the analyses are based is located in northeastern California, within the Lassen National Forest. The timber type is classed as pure pine, with more than 90 percent of the volume in ponderosa pine (*Pinus ponderosa* Dougl.) and Jeffrey pine (*P. jeffreyi* Oreg. Com.). The other timber species are white fir (*Abies concolor* Lindley and Gordon) and incense cedar (*Libocedrus decurrens* Torr.). The stand is all-aged, with young and overmature age classes well represented, but is understocked owing to light representation in the intervening age classes. With the exception of

small areas, the stand over the entire area appears uniform to the eye and not stratified according to any of the criteria used in mapping such stands. In other words, it appears to be a fairly homogeneous population.

The nine sections shown in figure 1 were selected for study because they were full sections and, with the exception of small areas, completely timbered. Board-foot volumes of trees 12 inches and more in diameter were totaled for the 2,304 individual 2.5-acre plots contained in the nine sections. These plots, rectangular in shape (2.5 by 10 chains), are designated as basic plots. In addition, because their

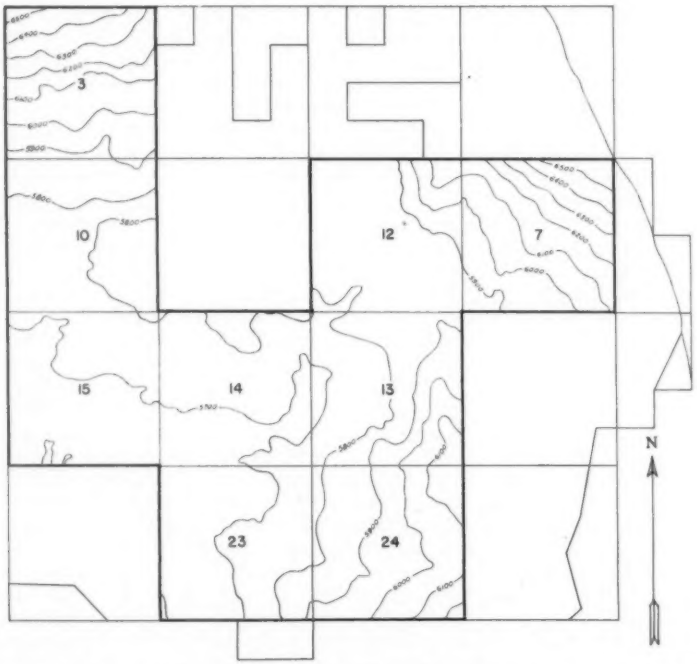

FIGURE 1.—Contour lines and section numbers of area selected for analysis from the Blacks Mountain Experimental Forest.

locations are mapped, adjacent plots may be combined into totals of 1,152 5-acre plots (5 by 10 chains), 576 10-acre plots (10 by 10 chains), or other shapes and sizes in multiples of the basic plot size and dimensions.

Some irregularity in size and shape of basic plots along section lines was unavoidable owing to variation of sections from exact mile squares. For these plots the volumes were proportionately reduced or increased to the 2.5-acre basis. The adjustments needed amounted to a negligible percent of the total. All basic plots were taken with the long axis in an east-west direction.

Subdivisions larger than plots are termed blocks, and are usually taken as regular Land Office subdivisions such as forties, quarter

sections, half sections, or sections. Figure 2 shows the size and shape of plots and blocks mainly used. When a plot making up part of a sample is selected independently and at random it constitutes a random sampling unit. When plots are spaced at regular intervals along lines, and the lines selected independently and at random, the line-plot combination is the sampling unit. Likewise, where plots are contiguous end to end forming strips and the strips are selected independently and at random, the strip constitutes the sampling unit. The resulting arrangements may be termed random line plot and random strip, respectively. When the sampling units are taken at

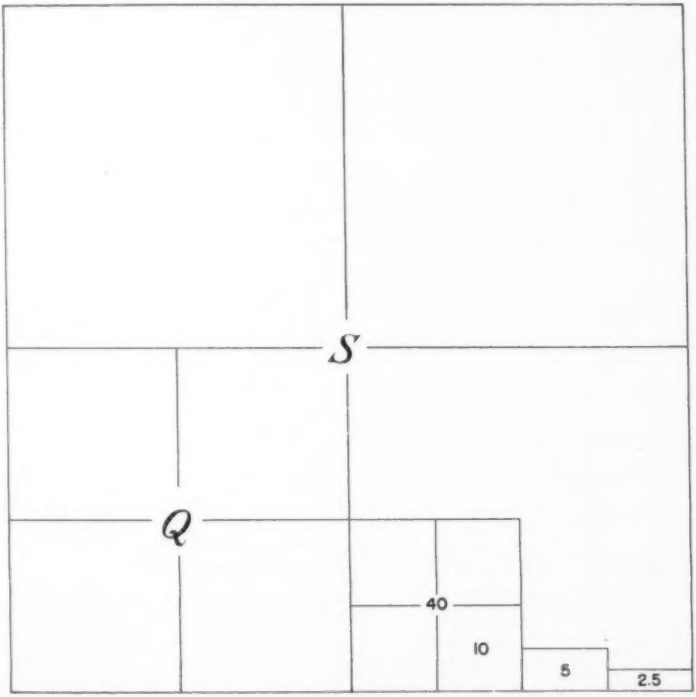

FIGURE 2.—Size and shape of plots and blocks: *S*, Section (640 acres); *Q*, quarter section (160 acres); smaller units designated by area in acres.

random from all possible in the population, the sampling is referred to simply as random, as contrasted to selection of equal numbers of units from each block, which is termed "*random within blocks.*" Systematic strip and line-plot arrangements are representative samples in that each block is sampled to the same intensity, but are not random.

The total variation between random sampling units is apportioned into parts contributed by various known sources and the error variance segregated by the analysis of variance method of Fisher. Descriptions of this method and others used in the study are given by Fisher (*2*, *3*) and Snedecor (*10*), and an instructive application of the method by Immer (*5*).

RESULTS

SIZE AND SHAPE OF PLOT

The essential information concerning stand variability is given in table 1. The mean square ratios all exceed F at 0.01 as given by Snedecor (*10*), and therefore volume is correlated with place and the population is heterogeneous. In general, variation between plot

TABLE 1.—*Analysis of variance*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F [1]	F at 0.01 [2]
Sections from general mean	109, 693. 3526	8	13, 711. 6691	6. 39	3. 26
Quarter sections within sections	57, 937. 4032	27	2, 145. 8297	3. 48	1. 98
Forties within quarter sections	66, 569. 5344	108	616. 3846	1. 65	1. 11
Tens within forties	161, 109. 7722	432	372. 9393	1. 76	1. 08
Fives within tens	121, 940. 8201	576	211. 7028	1. 83	1. 04
Basic plots within fives	133, 337. 7395	1, 152	115. 7446	------	------
Total, basic plots from general mean	650, 588. 6220	2, 303	[3] 282. 4961	------	------

[1] $F=\frac{\text{larger mean square}}{\text{smaller mean square}}$.

[2] As given by Snedecor (see text). Values equal to or exceeding these indicate that differences are highly significant statistically.

[3] $\sqrt{282.4961}=16.81$ = standard deviation of a basic plot from the population mean of 40.83 thousand feet board measure.

volumes tends to increase as the distance between plots is increased. For this reason, size and shape of plot will be an important factor in efficiency of sampling. Two plots taken side by side will in general include less of the stand variation within their combined area and more will remain between such pairs than will be the case with pairs of separated plots. Thus variation between 5-acre plots will be greater than variation between 5-acre sampling units each made up of two 2.5-acre plots spaced apart along a line. Likewise, owing to correlation between adjacent plots, variation between 5-acre plots, expressed in terms of single 2.5-acre plots taken as parts of 5's, will be greater than variation between 2.5-acre plots as random sampling units. Less stand variation per unit of area is included within 5-acre plots as sampling units than within 2.5-acre sampling units.

The mean square between 2.5-acre plots in table 2 is the total mean square from table 1. It is the error variance of randomly selected 2.5-acre plots. The corresponding value for 5-acre plots is obtained from table 1 by pooling sums of squares and degrees of freedom from 5's within 10's up to and including sections from general mean. The quotient of these two summations—449.3926—is the mean square of randomly selected 5-acre plots, expressed in terms of single 2.5-acre plots. This is considerably larger than 282.4961, and the effect on sampling efficiency is evident from the following formula for variance of a mean:

$$\sigma_M^2=\frac{\sigma^2}{n} \qquad (1)$$

in which

σ_M^2 = the variance of a sample mean, or the standard error squared
σ^2 = the variance, or mean square, of the basic plots
n = the number of basic plots.

For a given standard error, n will need to be 449.3926/282.4961=1.59 times as great with 5-acre plots as with 2.5-acre plots. In random sampling from an unlimited population, 1.59 times as much land is needed in sample area with 5-acre plots as with 2.5-acre plots to assure the same precision.

Stated in another way, 282.4961/449.3926=62.86 percent, the efficiency of 5-acre plots as compared to 2.5-acre. To equal in precision the estimate of volume based on sampling with 5-acre plots, an area only 62.86 percent as large would be needed with the smaller plots. The advantage of 2.5-acre plots over 10-acre plots is still greater.

When plots and sampling units larger than the basic plots are used, the variance is then weighted according to the number of basic plots in the larger plot. Likewise n is in terms of basic plots. Obviously, the resulting standard error obtained is not affected. To keep mean squares directly comparable regardless of size, shape, or arrangement of plots used, they are expressed in terms of single basic plots throughout this article.

It is evident from table 2 that, among the 10-acre plots, the long, narrow shape is the most efficient. This is due partly to the presence of small nontimbered areas of such shape that several 10 by 10 and 5 by 20 plots contained little or no volume and consequently increased the average variation. The effect of placing of plots with respect

TABLE 2.—*Relative efficiency of plots of varying size and shape in the use of land*

Plot size and shape	Mean square	Efficiency	Relative size of sample for a given precision of estimate
		Percent	
2.5-acre: 2.5 by 10 chains [1]	282.4961	100.00	1.00
5-acre: 5 by 10 chains	449.3926	62.86	1.59
10-acre:			
2.5 by 40 chains	[2] 566.0602	49.90	2.00
5 by 20 chains	[2] 711.8291	39.68	2.52
10 by 10 chains	687.4958	41.09	2.43

[1] 1 chain=66 feet.
[2] Obtained by separate calculation of population variance. All other mean squares obtained from table 1 directly by pooling degrees of freedom and sums of squares.

to direction of greatest stand variation is illustrated in figure 1. Variation in timber stands is usually greatest at right angles to the contour lines. In collecting the present data, the longer plot axis always extended in an east-west direction. Thus, within sections having marked changes in elevation, sections 24 and 13 were cruised with plot length in the direction of greatest variation, section 7 at a 45-degree angle to greatest variation, and section 3 parallel to greatest variation. In this respect it would seem that sampling by sections about balances. Had length always been taken at right angles to the contours, the advantages of long, narrow plots would be greater, and possibly the 5 by 20 plots would appear as superior to the 10 by 10.

It should be emphasized that the results in table 2 are based on random sampling from a theoretically infinite parent population. In sampling methods discussed later, where corrections are made to the mean of the limited population and variation between blocks

eliminated, these relative efficiencies will not be exactly true, although quite close.

RANDOM PLOT AND RANDOM-WITHIN-BLOCK CRUISES

As previously stated, in sampling a heterogeneous population it is necessary to select the sampling units independently and at random to insure that the heterogeneous elements are represented in about their true proportion. Using 2.5-acre plots as sampling units, random sample estimates of variance will tend to approach 282.4961, the true variance. As the number of sample estimates is increased, their average will approach more closely to the true, and likewise, estimates from larger samples will be grouped closely about the true variance and closely in accord with the normal law of frequency of error. A valid sample estimate of variance may be substituted in formula (1), in which n is known and the standard error calculated. This tells us the range from the sample mean within which the chances are 2 in 3 that the true or population mean lies. By doubling the standard error obtained we have the range for which the chances are 19 in 20.

With intensive sampling, formula (1) gives appreciable overestimates of sampling error because it estimates the range from the theoretical infinite parent-population mean while here we are interested in range from a parent population limited to 2,304 2.5-acre plots. By substituting 2,304 as n in the formula we can get the estimated range of the population mean from the limited population mean. This is the irrevelant portion of total variance of sample means and is represented by $\frac{\sigma^2}{N}$ in the following formula:

$$\sigma_M^2=\frac{\sigma^2}{n}-\frac{\sigma^2}{N} \qquad (2)$$

in which

N=the number of 2.5-acre plots in the limited population (a constant=2,304).

In practice a single estimate of variance together with the sample mean are the two statistics supplied. With the present data the true variance and mean are known for the limited population, and by varying n in formula (2) the expected grouping of sample means about the true mean can be calculated for different intensities of sampling. Since σ^2 changes with varying size and shape of plot, the effect of these factors is also reflected in the expected grouping of sample means. The appropriate mean squares for substitution as σ^2 in formula (2) are given in table 3, the values for which are derived from table 1. The expected standard errors for random samples of varying intensity, made up of plots of varying size, are given in table 4. In all, the expected standard errors based on true variance are shown for seven such cruises by the random arrangement of plots. For each cruise, four independent samples were drawn to compare expected values with observed values. The 2,304 plots were numbered consecutively and the required number of plots for each cruise selected by use of Tippett's (*11*) random sampling numbers. The sample

estimates of standard error and deviations from the true mean are shown in table 4 along with the corresponding true standard error.

TABLE 3.—*Variance of plots from the general mean and from block means*

Plot size and source of variation	Sum of squares	Degrees of freedom	Mean square [1]	Variance of plot means [2]	Standard deviation
2.5-acre:					
From general mean	650,588.6220	2,303	282.4961	282.4961	16.81
Within sections	540,895.2694	2,295	235.6842	235.6842	15.35
Within quarter sections	482,957.8662	2,268	212.9444	212.9444	14.59
Within forties	416,388.3318	2,160	192.7724	192.7724	13.88
5-acre:					
From general mean	517,250.8825	1,151	449.3926	224.6963	14.99
Within sections	407,557.5299	1,143	356.5083	178.2842	13.35
Within quarter sections	349,020.1267	1,116	313.2797	156.6398	12.52
10-acre:					
From general mean	395,310.0624	575	687.4958	171.8739	13.11
Within sections	285,616.7098	567	503.7332	125.9333	11.22
Within quarter sections	227,679.3066	540	421.6283	105.4071	10.27

[1] Variance of a single basic (2.5 acre) plot.
[2] Variance of the mean of basic plots making up larger plots.

TABLE 4.—*Expected and observed deviations of sample means from the true mean volume* [1]

Plot and sample size	Row No.	Random sampling from total population			Random sampling within blocks [2]		
		Standard error based on population variance	Standard error based on sample estimate of population variance	Observed deviation of sample mean from the true mean	Standard error based on population variance	Standard error based on sample estimate of population variance	Observed deviation of sample mean from the true mean
		Percent of 40.83	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*
2.5-acre:							
	1		4.85	−4.38		4.36	+9.18
3⅛-percent	2	4.77	4.95	+1.89	4.14	4.26	−.12
	3		4.87	+1.10		4.36	−3.58
	4		4.92	−3.11		4.38	+1.86
	5		3.11	+4.16		2.64	−.83
6¼-percent	6	3.32	3.16	−.81	2.88	2.82	+.37
	7		3.31	−1.03		3.60	+3.87
	8		3.40	−1.47		2.91	+3.08
	9		2.28	+1.86		1.62	−2.08
12½-percent	10	2.27	2.38	+1.79	1.87	1.69	+2.91
	11		2.23	−2.89		1.66	−1.08
	12		2.20	−.42		1.69	−1.40
5-acre:							
	13		3.99	−3.16		4.14	+3.80
6¼-percent	14	4.18	4.19	+9.58	3.50	3.92	−3.92
	15		3.53	+1.08		3.82	−3.70
	16		4.51	−1.49		3.13	−1.10
	17		2.86	+2.47		2.60	+.86
12½-percent	18	2.86	2.89	+1.74	2.39	2.57	+.44
	19		2.77	−5.85		2.30	−.17
	20		2.86	−2.50		2.67	+4.56
10-acre:							
	21		5.29	−1.10		3.50	−3.38
6¼-percent	22	5.18	5.12	−6.81	4.43	4.38	+1.47
	23		5.31	+.51		4.14	+1.05
	24		4.41	+3.23		5.93	+1.79
	25		3.37	+1.59		2.62	+2.28
12½-percent	26	3.54	3.39	−.39	2.77	2.67	−1.69
	27		3.67	+4.58		2.84	+3.43
	28		3.62	+.34		2.94	−4.80

[1] The true mean volume of 2.5-acre plots is 40.83 thousand feet board measure.
[2] Equal numbers of plots selected at random from each block. Blocks are taken here as the smallest square unit of area, sampled equally and with a minimum of 2 plots.

By means of the analysis of variance, however, it may be possible to reduce these standard errors by changing the arrangement of plots to permit the breaking up of total sum of squares into parts contributed from known sources, i. e., into a part due to variation between blocks, and a part due to variation within blocks. Each block is sampled equally with the plots selected at random. To estimate the within mean square, a minimum of two plots to the block is required. For the present, blocks will be considered as the smallest square unit of area so sampled. Degrees of intensity of sampling will be introduced by variations in block and in plot sizes. This arrangement of plots will be contrasted with random selection of plots over the entire area, the latter selection being almost certain to result in an unequal sampling of blocks.

The number of degrees of freedom for the sum of squares due to variation between blocks will be one less than the number of blocks. For the squares due to variation within blocks, the number of degrees of freedom will be the number of sampling units minus the number of block means from which deviations are measured. An application of the *F* test shows in all cases that the between mean square is significantly the greater, a significant portion of variance irrelevant to sampling error being eliminated by using the within mean square as error variance instead of the total. This analysis is illustrated for quarter-section blocks with 2.5-acre plot size in table 5, using data from table 3 with the necessary additional computations.

TABLE 5.—*Analysis of variance for quarter-section blocks and 2.5-acre plots*

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*
Between quarter sections	167, 630. 7558	35	4, 789. 4502	22. 49
Within quarter sections	482, 957. 8662	2, 268	212. 9444	----------
Total	650, 588. 6220	2, 303	282. 4961	----------

This procedure is legitimate since mean square within blocks, treating each block as a population, is the same within the range of error of random sampling, regardless of differences in block means. The pooled estimates of mean square within all blocks is much more precise than the estimate from a single block, even when applied to a particular block, because of the larger number of degrees of freedom. The reduction in error variance gained by use of blocks of smaller sizes is made clear in table 3.

In making a 6¼-percent cruise with 2.5-acre plots as sampling units, by the random arrangement shown in figure 3, the error variance is 282.4961, and standard error 3.32 percent of the mean. By random within blocks, the 144 plots will average 4 to the quarter section, which is the block unit, as in figure 4 and table 5; error variance will be 212.9444; and the standard error will be reduced to 2.88 percent. All of the reductions in expected standard errors for random within blocks, as compared to random for total population in table 4, are due solely to differences in plot arrangement. For each standard error based on

population variance, four estimates based on actual samples are given and the corresponding deviations of sample means from the true mean.

The effect on sampling error of size, shape, arrangement of plots, and intensity of sampling is reflected in the standard errors as calculated. If the effect of these has been correctly determined—population heterogeneity having been successfully overcome either by randomization alone, or by partial elimination followed by randomization—the deviations of sample means from the true mean

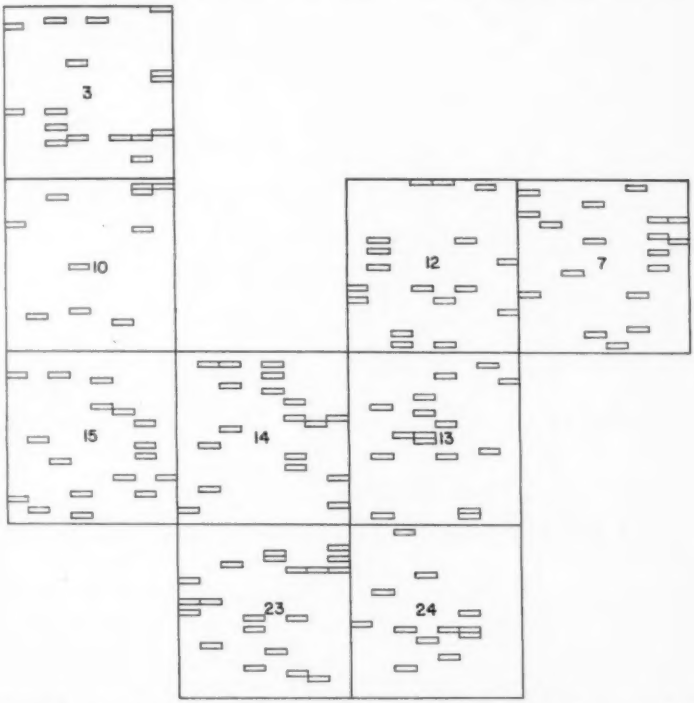

FIGURE 3.—Arrangement of 2.5-acre plots in a 6¼-percent random cruise of nine sections. Plots selected at random from the total population.

expressed in terms of standard units of the normal curve should not differ from a normal frequency distribution by more than may be attributed to error of random sampling alone. If the samples contain the information needed for assessing error due to sampling, the range of deviations in terms of standard units, arrived at from sample estimates of standard error, should likewise agree with expectation of normal grouping. That observed results agree with expected results is shown in table 6. The observed frequencies are within the range expected in 95 percent of such trials.

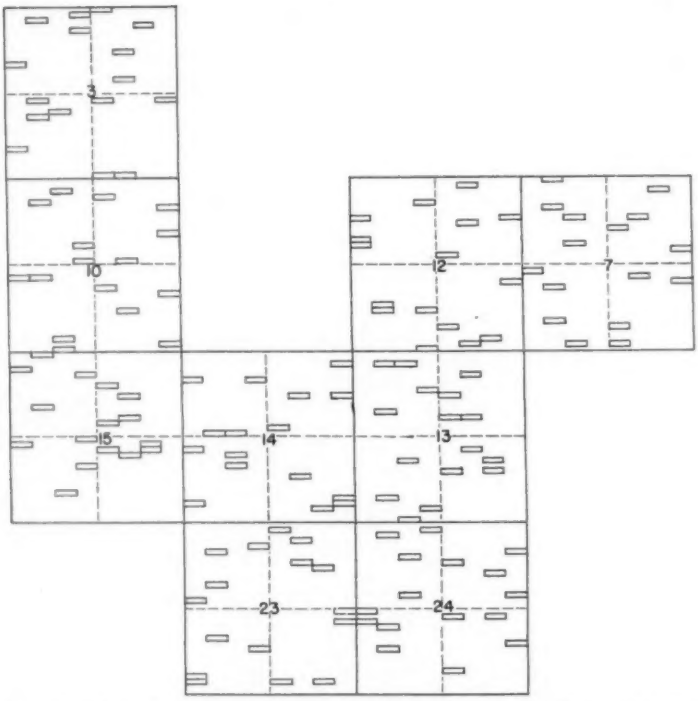

FIGURE 4.—Arrangement of 2.5-acre plots in a 6¼-percent random-within-block cruise of nine sections. Quarter section (40 by 40 chains) taken as the block unit.

TABLE 6.—*Expected and observed grouping of sample means about the true mean*

Range from zero difference in standard units of the normal curve	Expected frequency	Random, observed frequency		Random-within-block, observed frequency	
		Based on true standard error	Based on estimated standard error	Based on true standard error	Based on estimated standard error
Less than 0.253	5.6	7	6	5	4
0.253 to .524	5.6	6	7	6	7
.524 to .842	5.6	6	6	5	4
.842 to 1.282	5.6	5	4	7	8
1.282 and over	5.6	4	5	5	5
Total	28.0	28	28	28	28

STRIP CRUISES

Thus far the most efficient method of sampling indicated is the use of the random-within-block, or "stratified" arrangement of 2.5-acre plots, using blocks of such size that a minimum of two plots is taken

within each. A disadvantage of this arrangement is that it presents some difficulty in locating plots on the ground and in mapping all parts of the area to the desired standard. Within some blocks the plots are clustered together leaving large areas which could not be mapped without running additional line for that purpose alone. The irregularity of line running to locate plots may be partially overcome by use of the strips taken at random within blocks, as shown in figure

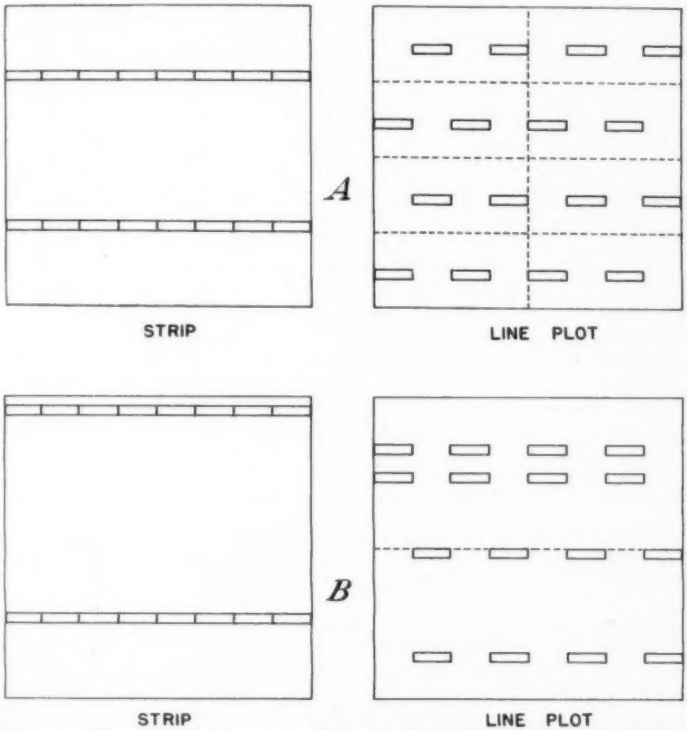

FIGURE 5.—Typical arrangements of plots in 6¼-percent strip and line-plot surveys: *A*, Systematic cruises; *B*, random-within-block cruises. Broken lines indicate block boundaries within sections.

5. Here the sampling unit is the strip, 2.5 by 80 chains, 2 of which are taken from the 32 possible in a section, the block unit. The mean square (in terms of a single 2.5-acre plot) between 28 such cruises is shown in table 7 as 385.5211. According to the previous reasoning, this differs by sampling error alone from mean square between strips within sections, which is estimated here as 425.1970. The *F* test fails to disprove this hypothesis.

TABLE 7.—*Analysis of variance based on strip cruises of 6¼-percent intensity*

RANDOM-WITHIN-BLOCK STRIP [1]

Source of variation	Sum of squares	Degrees of freedom	Mean square	F [2]	Variance associated with blocks
Between cruises	10,409.0688	27	385.5211	n. s.	
Sections within cruises	277,894.8542	224	1,240.6020	*2.92*	407.70
Strips within sections	107,149.6481	252	425.1970		
Subtotal	385,044.5023	476	808.9170		
Total, between strips	395,453.5711	503	786.1900		

POPULATION VARIANCE [3]

Between cruises			381.2952		
Sections	109,693.3526	8	13,711.6691	*35.96*	428.49
Strips within sections	106,381.3677	279	381.2952		
Total, between strips	216,074.7203	287	752.8736		

SYSTEMATIC STRIP [4]

Between cruises	2,835.2736	15	189.0182	2.21	
Sections within cruises	153,411.7346	128	1,198.5292	2.87	390.57
Strips within sections	60,104.5909	144	417.3930		
Subtotal	213,516.3255	272	784.9865		
Total, between strips	216,351.5991	287	753.8383		

[1] Mean volume of 28 cruises=40.79.
[2] n. s.=nonsignificant; values in italics exceed F at 0.01.
[3] True mean=40.83.
[4] Mean volume of 16 cruises=40.83.

True population mean square of strips within sections is 381.2952. Whether or not the estimate obtained differs significantly from this may be tested by the chi-square distribution:

$$\text{Chi square}=\frac{\text{Sum of squares}}{\sigma^2} \qquad (3)$$

For 252 degrees of freedom chi square is 209.5104 at $P=0.975$, and is 297.4360 at $P=0.025$. Substituting 381.2952 for σ^2, solving for each corresponding sum of squares, and dividing by 252 gives the range of mean square expected in 95 percent of such trials. The values obtained are 317.0052 and 450.0433. There is therefore no reason to suspect the sample estimate.

Since variation between sections is significantly greater than variation within sections, the use of total variance for error would give a serious overestimate, and not a valid one.

Systematic strip survey would seem to be quite comparable to strips taken at random within blocks as regards estimates of error mean square. Sixteen 6¼-percent cruises taken side by side include 100 percent of the area or the total population. Population variance of strip cruises is analyzed on this basis in the lower portion of table 7. Variation between such cruises is about one-half that between the random-within-blocks. This advantage in favor of the systematic

cruise is surprisingly large, even considering that such a cruise is more representative in that each half section is sampled equally, whereas in the random the smallest block is the section.

The variation between cruises is less than would be expected in 95 percent of such trials if the sampling were random. The use of the *F* test is not legitimate, however, because the strips are equidistant. The analysis of population variance is made to find how the components of total variance differ in the systematic arrangement as compared to random. Obviously total population variance between strips is the same for both arrangements—the two values shown here are not absolutely the same owing to the use of short-cut methods of computation with the cruise estimates. The component parts do differ. Variation associated with sections is less by systematic than by random, and variation of strips within sections is correspondingly greater. Systematic sample estimates of mean squares do not tend to the same values as do random, and the basis for segregating error mean square is not known. In a single cruise the information for doing this is not provided. With the random cruise, the plot arrangement determines the one and only basis supplied by the data for estimating error mean square, and a single cruise will provide this.

With intensive cruising of fairly uniform stands of timber, such as this, the sacrifice in precision of volume estimate by use of random strip as compared to systematic strip is considerable. In some cases it might be feasible to cruise scattered blocks by random strip until sufficient degrees of freedom are available for a satisfactory estimate of error variance, and then cruise the remainder on the systematic basis; this should give reasonable certainty that the more representative sampling will keep the estimate within the allowable range of error. The advantage gained in mapping is also a consideration favoring this. While for some areas suitable maps are available prior to the cruise, map making in connection with sampling is still an important consideration in most cruising.

LINE-PLOT CRUISES

In the random line-plot arrangement in figure 5, the random sampling unit consists of four 2.5-acre plots spaced equidistant across the section. Two such units are taken at random from the 32 possible in a half section, which is the block unit. The true variance between such cruises is 221.8812 (table 8). In random sampling the appropriate estimate of error is provided by mean square of line plots within half sections, which tends to the true value. The corresponding estimates obtained from 56 such cruises are within a range attributable to random sampling error, and do not differ significantly from each other.

TABLE 8.—*Analysis of variance in line-plot cruises of 6¼-percent intensity*

RANDOM-WITHIN-BLOCK LINE PLOT [1]

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F* [2]	Variance associated with blocks
Between cruises	10,109.0880	55	183.8016	n. s.	
Half sections within cruises	660,240.1175	952	693.5295	*5.05*	233.18
Line plots within half sections	228,976.3700	1,008	227.1591		
Subtotal	889,216.4875	1,960	453.6819		
Total, between line plots	899,325.5755	2,015	446.3154		
POPULATION VARIANCE [3]					
Between cruises			221.8812		
Half sections	128,774.5115	17	7,574.9713	*34.14*	236.72
Line plots within half sections	123,809.7229	558	221.8812		
Total, between line plots	252,584.2344	575	439.2769		
SYSTEMATIC LINE PLOT [4]					
Between cruises	3,106.6848	15	207.1152	1.21	
Half sections within cruises	177,375.0742	272	652.1142	2.60	200.88
Line plots within half sections	72,102.4804	288	250.3558		
Subtotal	249,477.5546	560	445.4956		
Total, between line plots	252,584.2394	575	439.2769		
Between cruises	3,106.6848	15	207.1152	1.06	
20 by 40 within cruises	394,693.3468	1,136	347.4413	1.58	64.10
Between plots within 20 by 40	252,558.0700	1,152	219.2344		
Subtotal	647,251.4168	2,288	282.8896		
Total, between plots	650,358.1016	2,303	282.3960		
POPULATION VARIANCE [5]					
Between cruises			221.8812	*1.10*	
20 by 40	202,289.9365	71	2,849.1540	*14.18*	89.04
Plots within 20 by 40	448,298.6856	2,232	200.8507		
Total between plots	650,588.6221	2,303	282.4961		

[1] Mean volume of 56 cruises = 40.70.
[2] n. s. = not significant; values in italics exceed *F* at 0.01.
[3] True mean = 40.83.
[4] Mean volume of 16 cruises = 40.83.
[5] True mean = 40.83.

A difficulty arises in connection with the systematic line-plot cruise shown in figure 5, in deciding upon the block unit, i. e., whether the arrangement is comparable to the random line plot or more comparable to random selection of two plots within blocks 20 by 40 chains in dimensions. In practice, with but one cruise supplying the total data, there is no way to determine by which means the most useful information will be obtained. With a population of 16 such cruises, it is possible to determine this for this particular area, but the results will not apply generally. When sampling units are selected independently and at random there is never any doubt as to the one valid basis for estimating error mean square.

If the systematic arrangement permits the same treatment as for the random line plot, the variance associated with blocks is less and mean square within is greater than the true for random sampling. As with the strip survey, such sample estimates tend to the true value for total variance, and variance between cruises is less than that of corresponding random cruises. The apportionment of total variance to sources within cruises is biased. The overestimate from one source is balanced by an underestimate from another source. This is still more pronounced when the 20 by 40 unit is taken as the block. The F test, if legitimate here, would show that both estimates of error mean square are in a range attributable to sampling. Of the two, mean square within 20 by 40's provides the more useful information. In other cases, where a single sample is taken, there would be no assurnace which if either would give a satisfactory estimate of error. The guess with strip survey was as logical as either choice here, and yet was shown to be of little use. In neither case is there any justification for use of total variance as error variance.

RELATIVE EFFICIENCY OF CRUISES

For a standard by which to measure relative efficiency of different cruises we may set up a range of 8.00 percent of the mean at $P=0.05$ for 3⅛-percent cruises. By doubling the intensity to 6¼ percent, we expect a reduction of error to 5.33 percent, and by doubling again to 12½ percent, an error of 4.00 percent. This follows from formula (1). The error mean square necessary to assure this precision is 199.9584 on a single 2.5-acre plot basis. Were the correction to limited population mean made, the efficiency would be greater, since the error mean square would then be reduced by multiplying by the proportion of population not in the sample—as is done by use of formula (2). This correction is not made in table 9 mean squares, since the relative efficiencies would not be changed greatly. As they are given, the effect of decrease of block size with increased intensity of cruise is segregated completely. The reciprocal of 199.9584, or invariance $=0.00500$, is taken as a unit of information per 2.5 acres and the different cruises rated on this basis. One-half unit indicates that sample size would need to be doubled to assure the same precision as that by a cruise supplying a full unit. If costs of cruising were the same per unit of area in the samples, use of the former method would double the cost.

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TABLE 9.—*Relative amounts of information by cruises*

Sampling unit and arrangement	Mean square	Invariance [1]	Units of information per 2.5 acres [2]
3⅛-PERCENT SAMPLING			
2.5-acre, 2 plots within quarter section	212.9444	0.00470	0.94
2.5 by 80-chain strips, random	752.8736	.00133	.27
4 2.5-acre plots equidistant along 80-chain line, 2 lines within section	252.0121	.00397	.79
6¼-PERCENT SAMPLING			
2.5-acre, 2 plots within 20 by 40	200.8507	0.00498	1.00
2.5 by 80-chain strips, 2 within section	381.2952	.00262	.52
4 2.5-acre plots equidistant along 80-chain line, 2 lines within half section	221.8812	.00451	.90
5-acre, 2 plots within quarter section	313.2797	.00319	.64
10-acre, 2 plots within half section	477.6623	.00209	.42
12½-PERCENT SAMPLING			
2.5 acre, 2 plots within forty	192.7724	0.00519	1.04
2.5 by 80-chain strips, 2 within half section	323.3341	.00309	.62
4 2.5-acre plots equidistant along 80-chain line, 2 lines within 20 by 80	189.3106	.00528	1.06
5-acre, 2 plots within 20 by 40	291.6305	.00343	.69
10-acre, 2 plots within quarter section	421.6283	.00237	.47

[1] Invariance $= \frac{1}{\text{mean square}} \cdot$ Value of 0.00500=1 unit of information.

[2] Based on 1 unit for 8.00-percent error at 0.05 level with 3⅛-percent cruises, 5.66-percent error with 6¼-percent cruises, and 4.00-percent error with 12½-percent cruises. Correction for limited population not included.

The random-within-block 2.5-acre plot cruises meet the standard of precision set up for each of the three different intensities. The random-within-block line-plot cruises are not far behind in the lower intensities and are slightly the more efficient in the 12½-percent intensity. Because of advantages in locating plots on the ground along with satisfactory sampling efficiency, the random-within-block line-plot arrangement of 2.5-acre plots appears to be the best selection.

For a given sampling unit, the increase in information with increased intensity of cruise, as given in table 9, is due to the decrease in block size and the consequent elimination of more variation irrelevant to sampling error.

The 100-percent inventory of the population in this case makes it possible to compare the range of error of systematic cruises with that of similar random cruises. The systematic cruises cannot be treated as were the random in table 9, but for 6¼-percent cruises, both strip and line plot, the true variation between means has been found for this limited population. In both cases the mean squares are less than for similar random cruises. This finding, together with the advantage in mapping, makes it appear doubtful whether these arrangements should be completely discarded simply because single samples do not provide the information needed for estimating sampling error. There are good possibilities that their sampling error can be closely approximated. Where maps are not needed, or are already available, a random cruise can be made in the same time as a comparable systematic cruise. The cruiser must decide whether the

added precision in estimating sampling error is offset by the loss in accuracy of volume estimate.

It has been previously explained[5] that the range of systematic sample means about the true corresponds satisfactorily with the range expected for random-within-block sampling in which square blocks are used. If a number of such blocks in a population were selected at random and cruised by a random arrangement, the pooled estimate of error variance so obtained would then be considered as satisfactorily applicable to the entire population sampled. The remaining blocks could then be cruised by a systematic arrangement, in which the same number, size, and shape of plots would be used. Discarding the restriction as to shape of blocks, blocks 40 by 20 chains (north-south by east-west dimensions) are suitable in the present study for a systematic strip arrangement as shown in figure 5. Random variation within such units tends to 195.5448. This compares very well with the mean square between cruises, 189.0182 in table 7. Obviously, however, this proposed method of approximating error variance could easily be carried beyond a reasonable point by the use of very short strip segments as plots and long, narrow blocks. Probably length of blocks should not exceed twice their width.

Where systematic line plots are taken, some such method of approximating error variance would appear quite safe. The mean square for error of random sampling with 20 by 40 blocks is 200.8507, and the mean square between systematic cruises is 207.1152. The justification for this approach to sampling error is apparent from inspection of the diagrams in figure 5. The systematic arrangements are more representative than the random. For line-plot sampling, each 20 by 20 is sampled by a plot; while in random sampling the plot locations may fall so that some 20 by 20's are not sampled at all and some are sampled with two plots.

F. Yates, chief statistician, Rothamsted Experimental Station, England, has suggested that the sampling error of systematic cruises in which a single sampling unit was taken in the center of each block, might be approximated by using error variance estimates from two randomly selected sampling units in blocks of the same size. Only sufficient random cruising would be done in a population to get an adequate estimate of variance within blocks, and the survey could be continued on the systematic basis. The gain in representativeness by the systematic arrangement as compared to random would then be reflected in reduction of error variance by use of blocks of just half the size needed with random cruises of equal intensity. With the 6¼-percent systematic strip cruise the mean square between the 16 cruises is 189.0182. The mean square between random strips within half sections is shown in the report previously cited as 323.3341. With only 15 degrees of freedom available for estimate, the difference is within a range attributable to error of sampling in an unlimited population. The value 189.0182 is true for the limited population only. The estimates from such cruises in other similar areas might well tend to something like 323.3341. There is no reason, for example, to expect that the strip arrangement should consistently give as

[5] HASEL, A. A. ANALYSIS OF SAMPLING METHODS FOR VOLUME DETERMINATION IN A PONDEROSA PINE FOREST. Calif. Forest and Range Expt. Sta. Prog. Rept. June 1937. [Multilithed.]

close or closer estimates of volume than the line plot, where the spacing apart of plots would appear to sample a block better than by a single strip of contiguous plots. Also the advantage over random strip should not be much greater than can be attributed to more representative sampling alone. It is suggested that this method of approximating error of systematic strip survey be used in preference to the ones previously suggested in which strip segments were considered as plots.

With line-plot arrangements the results by the two methods are quite alike. Variance between line-plot sampling units within 20 by 80's is 189.3106. This differs little from 200.8507, the variance between plots within 20 by 40's, and neither differs significantly from 207.1152, which is based on 15 degrees of freedom.

It has been noted that doubling of intensity, other factors remaining constant, reduces a sampling error of 8.00 percent to one of 5.66 percent. Extremely close estimation of sampling error and small changes in intensity based on estimates of error variance are not important, unless it is realized at the same time that biased error from various sources may contribute much the greater part of total error of cruise estimate. Time saved by lowering intensity can be very profitably spent on reduction of biased error.

PRECISION OF ESTIMATES OF ERROR VARIANCE

The mean squares taken as error variance have been based upon true values for the population dealt with. They are the values to which sample estimates tend. The range within which sample estimates group themselves about the true is dependent solely upon the number of degrees of freedom available for their estimate. The range of 95 percent of such estimates is given for the various random cruises in table 10, derived by use of formula (3). Where the estimate is based on as few as 8 or 9 degrees of freedom a single sample may easily give a very coarse basis for error calculation—not close enough for much reliance. In this respect random plots have a marked advantage over line plots, which in turn are better than strip. By use of a much smaller and probably more efficient size of plot than 2.5 acres, however, the ordinary intensity of cruise in a few sections will give an adequate basis for estimate. Such tests as are given in table 10 should be made the basis for decision as to the number of blocks that should be cruised by a random arrangement before continuing with the systematic as suggested previously.

TABLE 10.—*Range of sample estimates of variance*

Sampling unit and arrangement	Degrees of freedom	Mean square	Range of 95 percent of sample estimates	
				Percent
3⅛-PERCENT SAMPLING				
2.5-acre, 2 plots within quarter section	36	212.9444	123.8059– 319.2763	(−42)-(+ 50)
2.5 by 80-chain strips, random	8	752.8736	203.8405–1648.7932	(−73)-(+119)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within section	9	252.0121	74.4276– 532.1375	(−70)-(+111)
6¼-PERCENT SAMPLING				
2.5-acre, 2 plots within 20 by 40	72	200.8507	139.4796– 270.2647	(−31)-(+ 34)
2.5 by 80-chain strips, 2 within section	9	381.2952	112.6092– 805.1260	(−70)-(+111)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within half section	18	221.8812	100.4136– 388.2921	(−55)-(+ 75)
5-acre, 2 plots within quarter section	36	313.2797	182.1408– 469.7107	(−42)-(+ 50)
10-acre, 2 plots within half section	18	477.6623	216.1724– 835.9090	(−55)-(+ 75)
12½-PERCENT SAMPLING				
2.5-acre, 2 plots within 40	144	192.7724	150.2018– 239.0980	(−22)-(+ 24)
2.5 by 80-chain strips, 2 within half section	18	323.3341	146.3266– 565.8347	(−55)-(+ 75)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within 20 by 70	36	189.3106	110.0652– 283.8334	(−42)-(+ 50)
5-acre, 2 plots within 20 by 40	72	291.6305	202.5212– 392.4172	(−31)-(+ 34)
10-acre, 2 plots within quarter section	36	421.6283	245.1347– 632.1473	(−42)-(+ 50)

APPLICATION OF RESULTS

In cruising it is necessary to make compromises between what is theoretically correct and what is practically possible. Experienced cruisers would never consider using the random arrangement exclusively because it does not lend itself to map making, and the volume estimates are not as close as with the systematic arrangement. It is important, however, to have a valid and adequate estimate of error variance, and this is possible without a great initial sacrifice in time and money. Having that, it is possible to assure an estimate of the required precision so far as sampling error is concerned and by the cheapest method of cruise. Knowing sampling error, it is possible by later checks of estimate against actual cut to segregate error due to biased measurements, and direct efforts toward its reduction which are commensurate with its importance.

A timbered area to be cruised usually consists of several separate populations, which are segregated according to criteria used in mapping. In working out the method of cruise for a population, even though the stand appears uniform to the eye, heterogeneity of variation should be assumed. It follows then that size and shape of plot is an important consideration and tests should be made to find the kind of plot or sampling unit which includes the maximum of stand variation per unit of area. Obviously the minimum size of plot used in the present study is too large to give an indication of what minimum intensity of cruise is possible for a given range of error. As a rough guide until further studies are made, the minimum size with which plot volumes approach the normal type distribution is suggested, although the analysis of variance method does apply to definitely skewed distributions, which are not normal. At any rate the mini-

mum size tested should not often contain zero volume. The time required to cruise these basic plots and multiples of basic plots should be recorded, as well as travel and mapping time per unit of distance between plots. It may prove more economical in practice to use more area in fewer large plots than the theoretical minimum indicated with more and smaller plots. By selecting the plots at random within blocks additional needed information on initial intensity of sampling is gained. Plot arrangement and intensity will require further tests unless previous experience in such stands is available. A more intensive cruise than is believed necessary should be made by use of the random plot or line-plot arrangement within blocks of the minimum size that will be considered. If the stand is not patchy it will be possible to combine adjacent block units into larger blocks, and by pooling within and between sums of squares and degrees of freedom, to estimate variance within blocks of different sizes.

Advantage should be taken of the usual procedure of running cruise lines and orienting plots for the purpose of sampling more intensively in the direction of greatest variation. The Land Office subdivisions are convenient block units, and within sections the cruise lines are generally run north-south or east-west depending on direction of variation. By taking blocks of such size and shape that variation within is kept low, and that between correspondingly high, the maximum of variation irrelevant to sampling error is eliminated. When sufficient degrees of freedom have been built up to provide estimates of variance within a predetermined allowable range, the units of information supplied per basic plot may be calculated for varying arrangements and block sizes. By knowing the average man-hours required to travel to and cruise a plot by each method, the cost per unit of information will give the most efficient method of sampling by a random arrangement. If the cruise is continued with a systematic arrangement of the same intensity of cruise, the estimate of error will be high. A closer approximation can be made by reducing the size of block by a half, so that a single systematically placed sampling unit is in the center of each. Knowing from the preliminary work the variance of random sampling within blocks of this size, this estimate of within variance, when divided by the total number of blocks, or number of systematically placed plots, will give the approximate sampling variance of the mean. By extracting the square root the standard error of the cruise mean is obtained.

The above-suggested procedure for starting a cruise would not need to be repeated in similar stands, except to check on or improve error-variance estimates by cruising randomly selected blocks by the random arrangement. It is intended to apply to intensive cruising of stands which appear uniform within blocks of 40 acres or more, as in the pure ponderosa pine type. A procedure for random sampling within types occurring in small irregular patches is difficult to work out, particularly if a type map is not available beforehand. The same is true of extensive cruising, as in the Forest Survey in parts of the country where types change often. If these scattered small type areas were brought together, however, there is little doubt that analysis of variance would show that they too are heterogeneous populations and that total variance based on systematically spaced plots is not a valid

estimate of error variance. Such an estimate is useful only in that we can be sure that it is an overestimate.

Owing to the volume of timber inventory work in progress or in prospect, as in connection with preparation of timber-management plans and land-use studies, efforts to determine efficient methods of getting adequate samples in major timber types and for various stand conditions would be likely to yield results of considerable practical importance. Existing and proposed experimental forests are expected to represent fairly well the principal stands of timber within national forests. Complete inventories of these areas, with volumes recorded separately by sufficiently small units, would provide a good basis for working out sampling methods for the stands they represent.

CONCLUSIONS

The heterogeneous nature of stand variation within a 5,760-acre tract of the Blacks Mountain Experimental Forest is evidence that timber stands, even though they appear uniform to the eye, are similar to other soil crops in exhibiting systematic and yet partly disordered variation from point to point.

In a heterogeneous population, size and shape of plot are important factors in efficient sampling. A valid estimate of sampling error is possible only when the sampling units are selected independently and at random. By dividing the area into blocks of uniform size and shape, and selecting equal numbers and at least two random sampling units in each, a significant reduction in error variance is possible by Fisher's method of analysis of variance. The customary use of total variance as error variance and the statistical treatment of systematically spaced plots as random sampling units would be legitimate only if the population sampled were shown to be homogeneous. Such a condition seldom if ever exists. It is only by the use of random sampling that the elements contributing to heterogeneity are most likely to be represented in the sample in their true proportion.

Systematic cruises give closer estimates of volume than do similar random cruises and lend themselves better to map making. Since variation within separate blocks in a population varies within a range attributable to sampling error alone, and independently of the block means, only sufficient random cruising is suggested to assure an adequate estimate of error variance. If the remainder of the population is cruised by systematic spacing of sampling units, the estimate of error tends to be a little high. A closer approximation to sampling error is suggested if random variation within blocks of such size that they contain but one systematically placed sampling unit is used for error variance. In this way the gain in added representativeness of systematic sampling, as compared to random, is taken into account.

LITERATURE CITED

(1) Candy, R. H.
1927. accuracy of methods in estimating timber. Jour. Forestry 25: 164–169.

(2) Fisher, Ronald Aylmer.
1934. statistical methods for research workers. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London.

(3) Fisher, R. A., and Mackenzie, W. A.
1923. studies in crop variation. II. the manurial response of different potato varieties. Jour. Agr. Sci. [England] 13: [311]–320, illus.
(4) Goodspeed, Allen.
1934. a modified plot method of timber cruising applicable in southern new england. Jour. Forestry 32: 43–46.
(5) Immer, F. R.
1932. size and shape of plot in relation to field experiments with sugar beets. Jour. Agr. Research 44: 649–668, illus.
(6) Mudgett, Bruce D., and Gevorkiantz, S. R.
1934. reliability of forest surveys. Jour. Amer. Statis. Assoc. 29: 257–281, illus.
(7) Preston, John F.
1934. better cruising methods. Jour. Forestry 32: 876–878.
(8) Robertson, W. M.
1927. the line-plot system, its use and application. Jour. Forestry 25: 157–163.
(9) Schumacher, Francis X., and Bull, Henry.
1932. determination of the errors of estimate of a forest survey, with special reference to the bottom-land hardwood forest region. Jour. Agr. Research 45: 741–756, illus.
(10) Snedecor, George W.
1937. statistical methods applied to experiments in agriculture and biology. 341 pp., illus. Ames, Iowa.
(11) Tippett, L. H. C.
1927. random sampling numbers. 8 pp., 26 tables. London. (Tracts for Computers No. 15).
(12) Wright, W. G.
1925. variation in stand as factor in accuracy of estimate. Jour. Forestry 23: 600–607.

A STUDY OF PROTEIN EXTRACT FROM SOYBEANS WITH REFERENCE TO ITS USE IN FOOD[1]

By SYBIL WOODRUFF, *chief in foods*, EVELYN CHAMBERS, *graduate student*, and HELEN KLAAS, *first assistant in home economics, Illinois Agricultural Experiment Station*

INTRODUCTION

The soybean (*Soja max* (L.) Piper) is unique among plant foods in that it contains about 40 percent of protein. The supply of soybeans gives promise of being abundant, too; Illinois produced in 1937 a little less than 22 million bushels, an increase of about 27 percent over her crop for 1936. Here is a potentially important source of protein, little of which is now being consumed as food. Some people are advocating that the United States extend the food uses of soybeans for the sake of obtaining inexpensive protein. This might be done in either of two ways; by utilizing the beans themselves or by making from the beans a protein concentrate which could take its place with other protein-rich foods.

The purpose of this study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in the preparation of food.

REVIEW OF LITERATURE

The literature relating to soybean proteins and their physical behavior is not extensive and is somewhat confused by the variety of laboratory methods which have been used in extracting the proteins. Moreover, papers bearing on the topic have not been concerned with the uses of the protein in food technology but rather with its chemical nature. There is little in the literature concerning the methods that are being used for removing crude soybean protein for the manufacture of plastics. A recent paper by O'Brien (*10*)[2] gives methods and factory costs for such industrial operations.

Osborne included soybeans in his classic studies of vegetable proteins. In 1898 he and Campbell (*12*) suggested the name "glycinin" for the globulin they dissolved from soybeans with a 10-percent sodium chloride solution. This solvent has continued to be employed more often than any other in extracting globulins. The conventional definition of a globulin would exclude protein extracted by any other solvent than dilute neutral solutions of salts of strong bases with strong acids. Yet O'Hara and Saunders (*11*) have recently reported success in extracting crystalline protein having the characteristics of a globulin with either saturated or normal solutions of sodium chloride. They used orange seed, peanut, and other proteins, but their conclusion that the "ordinary text book definitions of globulins do not adequately consider the solubility properties of globulins" is concurred in by other writers who have worked with still other materials (*4*).

It has been commonly observed that soybean proteins are readily soluble in water. Osborne and Campbell (*12*) said the fact that as

[1] Received for publication March 11, 1938; issued December 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 746.

much as 16 percent of the glycinin could be dissolved in water was due to the presence of potassium phosphates in the seed. Both Nakajima (*9*) and Muramatsu (*8*) showed that if soybeans were extracted first with water, there was little remaining protein which would dissolve further in sodium chloride or sodium hydroxide solutions. The last-named author has divided the water-soluble nitrogen of soybeans as follows: Globulins (largely glycinin), 84.25 percent; albumins, 5.36 percent; proteose and nonprotein nitrogen, the remainder.

The usual methods mentioned in the literature for recovering the protein in solid form are dialysis to remove salt and treatment of the extraction liquor with either ammonium sulphate or acid. Hartman and Cheng (*2*, *6*) gave the isoelectric point of soybean protein as pH 5.00. In almost every case reported in the literature the protein product has been dried with alcohol and ether; Hartman and Cheng (*5*) recommended methyl alcohol and ether.

Comments on solubility usually pertain to the ease of dissolving the protein from the seed, not to the solubility of the separated, dried protein. Denatured glycinin was said by Tadokoro and Yoshimura (*13*) to be most soluble in 0.1 to 0.25 normal sodium hydroxide though it was also soluble in several acids. They also found that heating soybeans caused a large percentage of their proteins to be less soluble in water and more soluble in alkaline solutions. Gortner (*3*) believed solubility of globulins to be merely peptization and governed by the ions present. Nevertheless the solubility of glycinin has been variously reported and likewise its ability to coagulate.

METHODS OF EXTRACTION

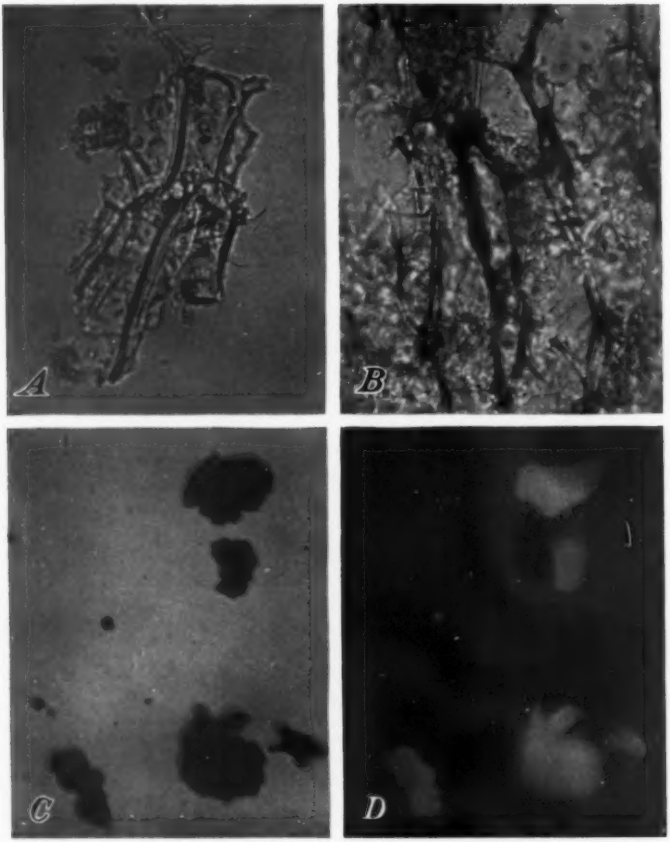
Mature soybeans of two "vegetable" types were used in the experiments, identified throughout the paper by serial Nos. 85666 and 81029. Both had been found in other work (*15*) with soybeans to be satisfactory for food uses. Without preliminary drying, each was ground in a Bauer mill to pass a 60-mesh screen; then extracted continuously for 24 hours with petroleum ether (boiling point 30° to 60° C.) and further ground in a porcelain mortar to pass a 100-mesh sieve. Previous heating of the soybeans was avoided because it tended to make the proteins less soluble.

Commercial fat-free soybean flakes [3] were used for much of the routine experimenting with solvents and methods. They were made from No. 2 Illini soybeans and had been treated only with organic solvent.

SOLVENT

Water was found to be the most practical solvent for protein extraction from the standpoint of yield and purity of product, and time and equipment involved. To 100 g of ground fat-free soybeans was added 750 ml of distilled water, and the suspension was agitated gently in a mechanical shaking machine for 30 minutes at room temperature. It was next poured into a canvas bag and the water "solution" pressed from it in a tincture press. The soybean residue was twice more extracted with fresh portions of 750 ml of water. Suspended matter was separated from the extracts by centrifuging, and the resulting "solutions" became less opaque and viscous in succeeding extractions. Little success was attained in filtering the

[3] Obtained from the Glidden Co., Chicago.



Microscopic appearance of soybean materials: *A*, Fat-free soybean flakes, ground to pass a 60-mesh screen; *B*, residue of flakes after extractions were completed, showing some cells still intact; *C*, soybean protein substance mounted dry showing dense masses of material; *D*, same field as *C* examined between crossed Nicols Note the luminous character of the protein masses, probably anisotropic. ×900.

extract even with a special filter of acid-alkali-treated asbestos and paper pulp which had been recommended by Hartman and Cheng (*5*). The liquors of the different extractions were kept separate and each in turn was diluted to 800 ml for precipitation of protein.

The commercial flakes absorbed less water than the soybeans, hence only 500 ml of water was used for their first extraction and 400 ml for the second and third. The extracts were diluted to 500 ml before the protein was precipitated.

Solvents other than water were used in a series of preliminary trials with commercial soybean flakes. Solutions of sodium chloride, sodium carbonate, and sodium hydroxide were used in turn. Sodium chloride in 10-percent solution had been frequently used by other investigators; it might well be expected to be the ideal solvent for the principal proteins of soybean, thought to be globulin in nature. Salt solution was not found to be superior to water as a solvent for the purposes of this experiment.

PRECIPITATING AGENT

Dilute acetic acid (about 1 or 2 ml) was added to each water extract of protein, and when the acidity reached pH 5.0 the protein precipitated as a white cloud. Hydrochloric was found to be no more successful than acetic acid. The addition of sufficient calcium chloride to make the liquor 0.02 molar with respect to the salt, aided in precipitating the protein, but this method likewise had no advantage over that of precipitating it with acetic acid. Dialysis through collodion bags effected a satisfactory precipitation of protein from the liquor extracted with sodium chloride solution.

The precipitate settled readily, after which the waste liquor was decanted and centrifuged from it. Successive portions of 70-percent, 95-percent, and then absolute ethyl alcohol were used to wash and dry the protein. It was last washed with ether and worked to dryness on a porous plate. The product was a white, fine powder which showed no tendency to gumminess after it had thus been thoroughly dried. Drying only in air gave a horny substance. No special advantage was found in drying the material with methyl alcohol as recommended by Hartman and Cheng (*5*).

YIELDS

About 52.6 percent of all the nitrogen originally present in the fat-free soybeans was recovered when the protein substance was dissolved in water and precipitated by acetic acid in three successive treatments. A fourth extraction was found not to add significantly to the total yield. The amount of protein left behind in the residue was about 22 percent of the quantity originally present. The fraction that failed to precipitate when acetic acid was added was about 15 percent of the original soybean protein. Unaccountable loss amounted to about 10 percent of the total protein. This waste, thought to be due almost wholly to seepage of the finely ground soybeans through the canvas bag, could be reduced by grinding the beans to pass a 60-mesh screen instead of a 100-mesh. The saving thus effected compensated for the slightly lesser solubility of the protein of the more coarsely ground beans.

Photomicrographs of the original sample, ground to pass a 60-mesh screen, and of the same after it had been extracted three times with water show (pl. 1, *A* and *B*) that it was probably the presence of intact cells which interfered with complete solution of the protein contained

in them and not any inherent insoluble nature of a part of the protein. The photograph also illustrates well the location of the protein and other material in relation to the cells.

ANALYSES

The composition and therefore the purity of the protein substance was found on analysis to vary with different methods of extraction. Nitrogen was determined by the Kjeldahl method; the ashing temperature was 600° C.; samples were dried for moisture determinations at 80° C. in vacuo for 24 hours.

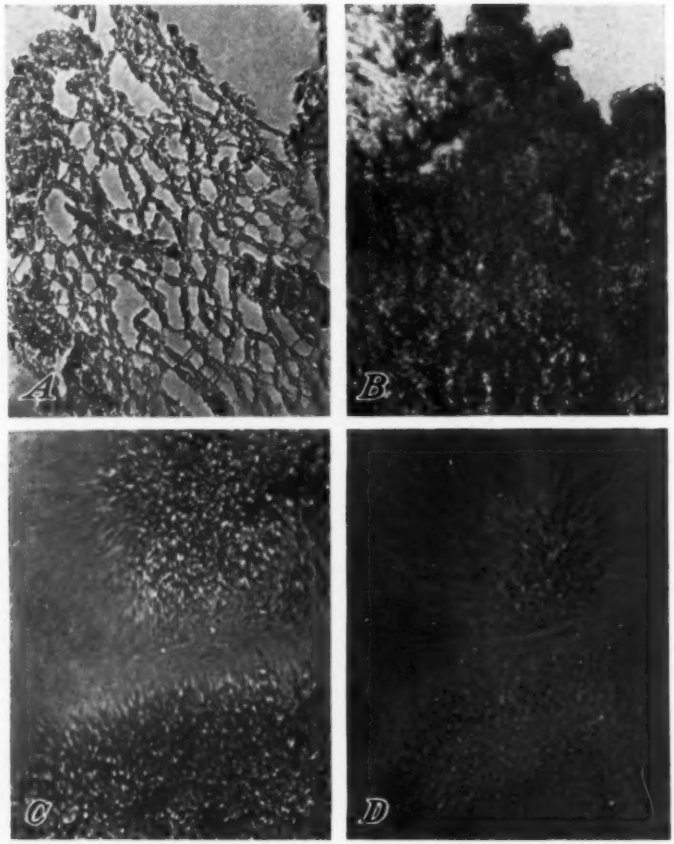
The protein substance yielded by dissolving with water and precipitating with acid, had the highest nitrogen content (16.13 percent) of all samples with the exception of the one dissolved in sodium chloride and dialyzed. The yield by the latter method was small, however. Other authors have reported the nitrogen content of protein substance, obtained by means of various solvents, to be from 12.25 to 17.53 percent. Data in table 1 show that samples obtained with other solvents were less exclusively protein in content; it was largely for this reason that water was selected as the most practicable solvent. The highest yield of protein substance ever obtained was with sodium hydroxide in 0.2-percent solution; but analysis of the material showed it to contain a high percentage of nonprotein constituents, particularly ash.

It is possible that hemicellulose material was dissolved by the alkali treatment just mentioned and was in turn precipitated by the acid used in separating the protein from solution. Occurrence of this kind may even have been observed to some degree when water was used as a solvent. The results given in table 2 show that 39 g of material out of the original 93.6 g of moisture-free beans could be accounted for in neither the protein substance nor the residue of soybeans left after three extractions were made. Of this 39 g of material unaccounted for, 22.78 g were shown by the data to be neither protein nor ash. It is assumed that this unidentified material dissolving out from the soybeans was largely carbohydrate in nature. It seems remarkable that the quantity of water-soluble constituents is as high as this. Further identification of water-soluble carbohydrates is under way.

TABLE 1.—*Analyses of protein substance extracted from soybeans by various methods*

Extraction method		Kind of soybeans	Protein substance		Constituents in moisture-free protein substance			
Solvent	Means of precipitating		Yield [1]	Moisture content	Ash	Nitrogen	Protein	
							N×5.71	N×6.25
			Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Water	Acetic acid	No. 85666 [2]	24.75	5.44	2.34	16.23	92.7	101.4
		No. 81029 [3]	27.37	6.00	2.05	16.13	92.1	100.8
		Commercial flakes. [4]	25.27	6.83	2.24	16.03	91.1	100.2
Sodium chloride solution, 10 percent.	Dialysis	do	10.35	5.78	.57	16.35	93.3	102.2
	Acetic acid	do	13.21	5.63	1.22	13.24	75.6	82.7
Sodium carbonate solution, 10 percent.	do	do	10.36	9.85	4.92	12.55	71.7	78.4
Sodium hydroxide solution, 0.2 percent.	do	do	35.58	4.98	1.21	13.57	77.5	84.8
	Hydrochloric acid.	do	19.83	7.36	1.79	13.00	74.2	81.2
Sodium hydroxide solution, 10 percent.	do	do	10.63	5.92	20.22	13.80	78.8	86.2

[1] From 100 g of fat-free soybeans.
[2] Average of 3 runs.
[3] Average of 2 runs.
[4] Average of 7 runs.



Microscopic appearance of protein substance during precipitation and during peptization: *A*, Threads or chains of newly precipitated protein substance in the mother liquor; *B*, dried protein substance mounted in water, photographed after 10 minutes to show that no peptization had occurred; *C*, dried protein substance mounted in 0.025-percent NaOH solution, after 1 minute standing, shown after peptization had started; *D*, same field as *C* after 10 minutes standing when most of the material had peptized. × 900.

TABLE 2.—*Nonprotein constituents removed from soybeans by extraction with water*

[Amounts based on 100 g of fat-free soybeans No. 85666]

Material	Air-dried weight	Moisture-free weight	Constituents present		
			Ash	Proteins N×5.71	Others by difference
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Fat-free soybeans	100	93.6	6.32	45.11	42.16
Protein substance	24.7	23.4	.55	21.69	1.16
Residue	34.0	31.2	.79	12.20	18.22
Loss in waste liquor, etc.		39.0	4.98	[1] 11.22	22.78

[1] Analysis of the waste liquor accounted for 6.05 g of this protein loss.

PROTEIN CONVERSION FACTOR

In recording the protein equivalent of the nitrogen percentages found by analysis, the factor 5.71 has been employed, since Jones (7) found it to be the correct one for soybeans. Few investigators even mention the conversion factor they have used, though it appears likely from their results that usually they have used 6.25. That the factor 6.25 was incorrect for the nitrogen yields of this study is indicated in table 1, where the protein content of the substance is shown as computed with each factor. The factor 6.25 made the protein appear to be more than 100 percent of the weight of substance. The protein content of the moisture-free protein substance was 92 percent (N×5.71).

SOYBEAN PROTEIN SUBSTANCE

The general physical behavior of the protein substance was a matter of major concern in the study because the usefulness in food technology of such a concentrated form of protein would be determined by properties like solubility, ease of coagulation, and behavior as a colloid. Several tests designed to reveal its potential functioning in this food capacity were carried out. Samples extracted from the three varieties of soybeans showed no recognizable differences in general behavior.

Separation and identification of the proteins which were precipitated from the water extractions was not one of the purposes of this study. The term "protein substance" has been used by the authors to refer to the recovered material because it was thought unlikely that only an individual protein was present. If other writers are correct, the protein substance was largely the globulin glycinin. It contained, as the above-mentioned analyses indicated, some moisture, a small amount of ash, and about 5 percent of unidentified material.

APPEARANCE AND SOLUBILITY

The protein substance was a tasteless, odorless white powder, the presence of which would be wholly unobjectionable as an addition to foods. So far as could be told, it was not crystalline. During the process of its precipitation from the liquor, microscopic threadlike chains or networks were characteristically present. Such an appearance is shown in plate 2, *A*. During drying the clumps assumed an appearance which would have been described as amorphous except

for the fact that under the microscope they were luminous when examined dry between crossed Nicols. It seemed possible that each clump was made up of innumerable anisotropic units; their luminosity could not be extinguished as the Nicols were turned, but this might have been due to the countless anisotropic units assuming as many different positions. Plate 1, *C* and *D*, shows masses of protein substance mounted dry and examined without and with crossed Nicols.

In water, the clumps exhibited no change in microscopic appearance which could be attributed to dissolving action, though anisotropy did not exist. This is shown in plate 2, *B*, where the clumps remained unchanged after standing in water for 10 minutes. The substance was seen to disintegrate rapidly when it was suspended in a very dilute sodium hydroxide solution (0.025 percent) and watched for several minutes under the microscope. Plate 2, *C* and *D*, shows that the protein clumps were peptizing even after standing 1 minute in the dilute alkali and had almost disappeared after 10 minutes.[4]

But little of the protein substance could be redissolved in water or even in sodium chloride solution. This was true while the material was still moist and also after it had been dried with alcohol. The protein substance was suspended in the solvent and shaken at room temperature for 1.5 hours, at the end of which time only about 12 to 20 percent of it had disappeared. It gave no appearance of dissolving in water though it was peptized readily by either 10-percent sodium carbonate or 0.025-percent sodium hydroxide solutions. The globulin had obviously undergone a transformation to an insoluble protein during the precipitation process. The protein may well have been further denatured during drying, but whether it was greatly exaggerated by alcohol treatment, was not determined.

Both 10-percent sodium carbonate and 6 N acetic acid solutions peptized or softened the protein to a gelatinous mass in a short time. Either of the last two solvents might safely be used in less concentrated solutions for such purpose in food preparation. The dried protein substance, originally extracted from the soybeans with alkaline solutions, dissolved to a somewhat greater extent than the one obtained by the method adopted. But this fact was probably explained by the presence of significant amounts of nonprotein impurities in the protein substance obtained by such solvents.

FOAMING ABILITY

The marked tendency of soybean suspensions to foam was a troublesome property in the processes of extraction, but on the other hand, it was seen to have certain possibilities of practical application. Several tests were made to determine whether the protein substance itself had any foaming ability with the idea in view that it might be put to use in food technology wherever egg white or gelatin are now employed for such purposes.

Eight grams of material, either ground soybeans, or protein substance, or residue from the extractions, in 100 ml of water was whipped at high speed in an electric mixer for 8 minutes. The volume of the foam if one formed at all was measured and then it was emptied into a large funnel containing two layers of cheesecloth. The volume of fluid which had drained from the foam at stated intervals was meas-

[4] Acknowledgment is made of the assistance of Dr. Majel M. MacMasters, associate in home economics, in the preparation of the photomicrographs.

ured as an indication of foam stability, more liquid draining from the less stable foam. The foaming ability of the liquors was measured by treating 100 ml of the liquor as above. The results of the foaming test are given in table 3.

TABLE 3.—*Volume and stability of soybean liquor foams*

Material	pH of solution	Volume of foam [1]	Water drained from foam [1] in—		Remarks
			5 minutes	30 minutes	
		Milliliter	*Milliliter*	*Milliliter*	
Fat-free soybean [2]		600	4	28	Heavy foam, thick consistency.
Residue, moist [2]	6.0	250	70		Thick paste, little foam.
Residue, dried [2]		(3)			
Protein substance:					
Used while still moist [2]		(3)			
Used after dried [2]		(3)			
Protein liquors, first extractions:					
Before precipitation of protein	6.1	1,200	0	60	Stable foam.
After precipitation of protein	5.0	1,600	2	60	Stiff, dry foam.
After precipitation of protein and addition of Na_2CO_3.	6.0	1,400	4	65	Foam like egg white.
After precipitation of protein, followed by removal of heat-coagulable proteins.	5.0	1,700	40	80	Do.
Protein liquors:					
First extraction, after precipitation	5.0	1,600	5	70	Stiff, dry foam.
Second extraction, after precipitation	5.0	1,100	40	85	Stiff foam.
Third extraction, after precipitation	5.2	500	75	85	Thin, watery foam.

[1] Foam produced by 100 ml of solution or suspension.
[2] A suspension contained the equivalent of 8 g of air-dry material per 100 ml.
[3] No foam.

Liquors remaining after most of the protein substance had been precipitated produced fairly stable foams of large volume. One such sample also became ropy and gelatinous after standing overnight in the refrigerator. The presence of mucilaginous constituents has commonly been observed in this laboratory in both green and mature soybeans. In fact, the liquor present in canned green beans, processed at 10 pounds pressure, has usually been found to appear as a soft jelly. No one pH value seemed to be required for the foaming, and the amount of protein dissolved in the liquor did not determine the volume of the foam. With a diminution in amounts of all constituents in succeeding extractions, the volume and stability both decreased.

The soybean residue from the extractions had practically no foaming power in spite of the fact that it still contained about 37 g of protein in 100 g. of moisture-free material. The original fat-free soybeans, on the other hand, formed a fairly stable foam of small volume; part of its stability seemed to be due to suspended matter, for its consistency was very different from that of the other foams.

The results showed that the protein substance, having undergone transformation to an insoluble form during its precipitation, had no ability to produce a foam either when used as freshly precipitated, still moist material or when suspended in water again after it had been dried. Watts (*14*), who investigated the whipping ability of ground, defatted soybeans, had attributed the foams obtained to protein contained by the flour. It is the opinion of the authors that other soybean constituents, e. g., saponins, pectinous or gumlike carbohydrate

derivatives, are responsible to a great degree for the foaming of the ground soybeans and of the liquors. Carbohydrates and like constituents are in process of investigation in the authors' laboratory now. The presence of saponins in soybeans has been reported by several groups of investigators, one of whom, Burrell and Walter (*1*), have recently prepared a crystalline saponin from soybean meal and studied its behavior.

OTHER PHYSICAL PROPERTIES

The protein substance differed from ground fat-free soybeans in several points of behavior. For one thing, it failed to absorb water when moistened and allowed to stand. One of the outstanding characteristics of soybean flour is its ability to absorb and hold large amounts of water. For example, a commercial low-fat soybean flour has been found to take up about 2.5 times its weight of water. This was determined by centrifuging off at high speed all surplus water which the moistened flour failed to retain. By the same test the residue of soybeans left after the proteins were extracted was found to have doubled the original capacity of soybeans to hold water. Milliliters of water absorbed by 100 g of material were as follows:

	Milliliters
Commercial soybean flour, low-fat	245
Commercial soybean flakes, fat-free	255
No. 85666 soybeans, fat-free	225
Residue from No. 85666 soybeans after protein extraction	490

These results indicated that other constituents, probably carbohydrate in nature, were responsible to a large degree for the swelling of soybean products in water.

Seed globulins have usually been reported in the literature to be not heat-coagulable. There is evidence of their slow rate of coagulating in the fact that "soybean milk" can be boiled for several minutes without visible change. Soybean milk is the name given a milky-white, watery suspension which is in common use in the Orient. Tests on the water extracts obtained in these experiments showed that the protein present did not coagulate by heat so long as the pH value was 6.0. After an addition of acetic acid had reduced the pH value to 5.00 and the precipitated protein had been removed, the remaining liquor allowed successive coagula to form at 66°, 80°, and 96° C. as heating and filtering were continued. But in all, the weight of heat-coagulated proteins was only about one-fifteenth of that of the protein known from the analyses to have been present in the liquor.

The very slight solubility of the protein substance has been mentioned already. The small amount which did dissolve, either as freshly precipitated or previously dried protein, showed no visible coagulation even when heated to boiling.

VALUE AS A PROTEIN SUBSTITUTE IN COOKERY

The protein substance being a tasteless white powder was found to have no objectionable features as an article of food. Several tests were made for the purpose of determining whether it had advantages in behavior as a colloid. A few typical dishes were prepared to learn whether it could be satisfactorily substituted for the usual weight of egg protein used for thickening purposes. In a custard no thickening whatever was observed whether the soybean protein was boiled with

the milk or allowed to cook at the lower temperature of the usual baked custard. This was true when both dried and freshly precipitated protein substance were used. Salts or other constituents of the milk, therefore, did not favor peptization of the soybean protein. Muffins were made to contain egg protein, or soybean protein substance in both dried and freshly precipitated forms, or no protein of either kind. The products made with soybean protein were neither better nor worse than those made with no egg at all. The ones containing egg were superior, however, to those without egg. Soybean flour has only slight thickening or binding quality, hence but little of this property has been lost in the conversion of flour to protein substance.

The soybean protein was found to have two advantages over soybean flour; it contained more than twice as much protein and it was free from flavor. Even though it was not found to be a successful substitute for animal proteins for cooking purposes, its presence in a mixture did not interfere with the expected reactions of other components. There is no reason to believe that its digestibility and utilization in the body have been lessened by converting it into a difficultly soluble form of protein during the process of extracting it from the soybean.

It is suggested that soybean protein might be incorporated with any food of moderately thick consistency if there is an advantage in thus increasing the protein content of the diet. Cereals, thickened soups, vegetable dishes, and escalloped foods of many kinds might have the protein substance incorporated in rather large amounts. Further work may reveal ways of reducing the amount of denaturation of the protein as it is precipitated, dried, and stored. Possibly it might then be used still more effectively in food preparation.

SUMMARY

The purpose of the study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in food preparation.

Protein was extracted from two varieties of soybeans and from commercial soybean flakes by treating fat-free finely ground beans with water at room temperature and then precipitating the protein from the extract by acidification with acetic acid to pH 5.0.

The dried protein substance thus obtained represented about 52.6 percent of the nitrogen originally present in the soybeans. It was 92 percent protein ($N \times 5.71$) on a moisture-free basis.

The protein substance was not crystalline but appeared to be anisotropic. Photomicrographs show its luminous appearance between crossed Nicols and also show it in the process of precipitating and peptizing.

It was not measurably soluble in water or salt solution, though acetic acid and sodium carbonate solutions caused it to soften and swell. Suspensions of it did not foam; in this behavior it differed markedly from suspensions of ground fat-free soybeans, the foaming ability of which is probably due partly to nonprotein constituents.

The protein substance did not produce a thickening or binding of food ingredients similar to the effect caused by egg proteins in custards or muffins. Neither did its presence interfere with expected reactions of other components during cooking. It was tasteless and wholly unobjectionable as an addition to other foods.

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It has been suggested that the protein substance might be incorporated in many food dishes for the purpose of adding to their protein content. The substance is thought to offer possibilities as a new source of food protein, and to have the advantage over soybean flour of higher protein content and freedom from flavor.

LITERATURE CITED

(1) Burrell, R. C., and Walter, E. D.
1935. A saponin from the soy bean. Jour. Biol. Chem. 108: 55–60, illus.

(2) Cheng, Libin T., and Hartman, R. J.
1936. Studies on soy-bean proteins. I. A new method of preparing soy-bean proteins. Jour. Chinese Chem. Soc. 4: 149–151.

(3) Gortner, Ross Aiken.
1929. Outlines of biochemistry, the organic chemistry and the physico-chemical reactions of biologically important compounds and systems. 793 pp., illus. New York and London.

(4) ———, Hoffman, Walter F., and Sinclair, Walton B.
1929. The peptization of wheat flour proteins by inorganic salt solutions. Cereal Chem. 6: 1–17, illus.

(5) Hartman, R. J., and Cheng, Libin T.
1936. Studies on soy-bean proteins. II. An improved method for the preparation of glycinin. Jour. Chinese Chem. Soc. 4: 152–156.

(6) ——— and Cheng, Libin T.
1936. Isoelectric point of glycinin. Jour. Phys. Chem. 40: 453–459, illus.

(7) Jones, D. Breese.
1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U. S. Dept. Agr. Cir. 183, 22 pp.

(8) Muramatsu, Shunsuke.
1920. Chemical and physiological studies of the proteins of the soy bean. Jour. Tokyo Chem. Soc. 41: 311–354. [In Japanese. Title also in English.]

(9) Nakajima, Kenzo.
1932. Studies on the protein and oil of soy bean. Hokkaido Imp. Univ. Faculty Agr. Jour. 31: 165–356, illus.

(10) O'Brien, W. J.
1936. Soya bean proteins. Oil and Colour Trades Jour. 90: 1434–1436, 1442.

(11) O'Hara, L. P., and Saunders, Felix.
1937. Studies in proteins. VI. The solubility of the nitrogenous constituents of certain seeds in sodium chloride solutions. Jour. Amer. Chem. Soc. 59: 352–354.

(12) Osborne, Thomas B., and Campbell, George F.
1898. Proteids of the soy bean (glycine hispida). Jour. Amer. Chem. Soc. 20: 419–428.

(13) Tadokoro, T., and Yoshimura, K.
1928. Chemical studies on the denaturation of proteins. Hokkaido Imp. Univ. Faculty Agr. Jour. 25: 117–132. [Abstract in Chem. Abs. 23: 2996–2997. 1929.]

(14) Watts, Betty Monaghan.
1937. Whipping ability of soy-bean proteins. Indus. and Engin. Chem. 29: 1009–1011, illus.

(15) Woodruff, Sybil, and Klaas, Helen.
1938. A study of soy bean varieties with reference to their use as food. Ill. Agr. Expt. Sta. Bull. 443, pp. 425–467., illus.

LINKAGE OF THE Q B Gs GROUP IN SORGHUM[1]

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INTRODUCTION

A rather large number of papers on the genetics of sorghum (*Sorghum vulgare* Pers.) has been published during the past 20 years. The sorghum plant is well adapted to genetic studies because of its growth habits, diversity of characters, and relatively small number of chromosomes. The number of seeds on a single head is usually not less than 500 and may reach a total of 6,000. Most varieties, under suitable conditions, produce tillers and nodal branches and thus continue to bloom intermittently until the plants are killed by frost. So far as known, all varieties of sorghum, including broomcorn and Sudan grass, can be intercrossed freely, and the resulting hybrids are completely fertile.

The inheritance of approximately 50 factor pairs in sorghum has been determined by the writers during the past 12 years. The characters were investigated to determine their linkage relations, and it has not seemed necessary to publish detailed data on simple independent genetic factors. Many of these genetic results were listed, however, in summary tables in an article on sorghum improvement (*10*).[2] This paper reports a linkage group of three factors studied in the coupling phase and the independence of this group from several other factors that have been reported previously by the writers or by other workers. The linked factors are those for reddish and blackish plant color (*Qq*), presence and absence of brown nucellar layer (*Bb*), and normal green and a green-striped chlorophyll deficiency (*Gsgs*).

REVIEW OF LITERATURE

ASSOCIATED CHARACTERS

In 1930 Reed (*23*) reported seedling stem color to be simple in inheritance with red stems dominant to green. Working independently, Karper and Conner (*6*) shortly thereafter reported linkage between seedling stem color and a seedling albino with 41.34 percent crossing over. The factor pairs for this linkage were designated *Rr* and *Ww*, respectively. A two-factor segregation for seedling stem color was reported by Woodworth (*30*).

According to Rangaswami Ayyangar (*13*), Benson and Subba Rao in 1906 observed the association of dull leaf midrib with juicy stalks and white midrib with pithy stalks. Swanson and Parker (*28*) found a higher percentage of smutted plants in rows homozygous for juiciness

[1] Received for publication April 15, 1938; issued December 1938. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Texas Agricultural Experiment Station at Substation No. 12, Chillicothe, Tex. Technical Series No. 442, Texas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 756.

than in rows containing pithy-stalked plants, but they suggested that instead of linkage between smut susceptibility and juiciness there may have been physiological correlation between smut susceptibility and sweetness. These authors found a single-factor difference between dry and juicy stalks and used the symbols *D* and *d* for dominant dry and recessive juicy, respectively. Rangaswami Ayyangar et al. (*18*) used the same symbols in reporting the independent inheritance of juiciness and sweetness, and in a later paper (*17*) they reported a linkage between purple leaf-sheath color and juiciness of stalk with 30 percent crossing over in the F_2 repulsion phase. The factors for purple leaf sheath and its recessive allelomorph, brown leaf sheath, were designated *P* and *p*, respectively.

Rangaswami Ayyangar et al. (*20, 21*) have observed close association among colors of the anther, stigma, and grain. The associations were assumed to result from expressions of the same color factors. Sieglinger (*25*) found yellow stigmas and colored seed segregating as a unit with white stigmas and white seed, and assumed stigma color to be another expression of the factor for seed color or else due to a very closely linked gene with no crossing over in his populations.

Vinall and Cron (*29*) reported dominance of red glumes over black in the F_1 and a 3:1 segregation in the F_2. Rangaswami Ayyangar et al. (*19*) also found a single-factor segregation and called attention to the association between color of glumes and leaf-sheath color. They designated the factors for dominant reddish purple and recessive blackish purple by the symbols *Q* and *q* and illustrated the phenotypes in color (*19, pl. XLII*). Rangaswami Ayyangar et al. (*16*) reported a linkage between the factor pair *Qq* and one of two factors for brown in dry anthers and grain which they designated B_1 and B_2 (*22*). This linkage was studied in the repulsion phase and no crossing over was observed in F_2 and F_3 populations totaling about 10,000 individuals. Recently, Rangaswami Ayyangar (*14*) reported a linkage between the *q* factor and the genes controlling the presence or absence of the nucellar layer, but at this writing has given no data indicating the intensity of the linkage.

Karper and Conner (*6*) reported a lethal yellow seedling character recessive to normal green and designated the factor pair Y_2y_2. Karper (*5*) found starchy endosperm acting as a simple dominant to waxy with the F_2 seeds on F_1 heads segregating 3:1 and F_1 pollen segregating 1:1. The symbols *Wx* and *wx* were used for the factor pair. Karper et al. (*7*) reported a linkage of the factor pairs Y_2y_2 and *Wxwx* with 26.5 percent crossing over.

MISCELLANEOUS INDEPENDENT CHARACTERS

The symbols *A* and *a* have been used to designate the pair of factors for awnless and awned, respectively. Vinall and Cron (*29*) found a segregation of 3 awnless to 1 awned in the F_2 of a Dwarf milo × feterita cross, but Sieglinger et al. (*26*) pointed out that the segregation in this particular cross is 1 tip-awned : 2 weak-awned : 1 strong-awned. Ramanathan (*12*) reported 3 short-awned to 1 long-awned, and Sieglinger (*25*) found 3 awnless to 1 awned in a Blackhull kafir × darso cross. In this cross Sieglinger observed independent inheritance of awns and seed color.

Karper and Conner (*6*) reported a virescent yellow seedling which occurred in F_2 populations in the ratio of 3 green to 1 virescent yellow. The factor pair concerned was designated V_2v_2.

Karper and Stephens (*8*) reported normal flowers dominant to antherless with a 3:1 segregation in the F_2, and Martin (*10*) used the symbols *Al* and *al*, respectively, to designate the factor pair involved. Karper and Stephens also gave the inheritance of twin-seeded spikelets, resulting from full development of the lower normally sterile flowers, as simple dominant to single-seeded spikelets. No symbols were used for the factor pair.

SEED COLOR CHARACTERS

Seed colors have been studied more intensively in India than in America, and several factors have been reported by workers in India which have not been mentioned in papers published in this country. Graham (*4*) found red (*R*), yellow (*Y*), and white an allelomorphic series except that some whites were undeveloped reds requiring the presence of yellow for expression of color. Graham's hypothesis was revised by Rangaswami Ayyangar et al. (*20, p. 603*). In this revision the authors assumed the following factors for color of sorghum grains:

Red	*YYRRWWII*
Pink	*YYRRWWii*
Yellow	*YYrrWWII* *YYrrWWii*
White (red base)	*YYRRwwII* *YYRRwwii*
White (yellow base)	*YYrrwwII* *YYrrwwii*

A basic yellow plant color is represented by the symbols *YY* but apparently no allelomorph has been found. A factor pair for red grains was designated *Rr*. The factor *R* masks yellow and when *R* is present the grains are red, pink, or white depending on additional factors for intensity of color and wholeness of color. The allelomorph, *r*, permits expression of yellow. Red-grained plants have a color-intensity factor assigned the symbol *I*, but potentially red-grained plants are pink-grained when only the allelomorph, *i*, is present. A factor pair for wholeness of color represented by the symbols *Ww* determines whether the seeds are colored or white. When the factor *W* is present the seeds are red, pink, or yellow; but when only the allelomorph, *w*, is present the seeds are white except for a slight expression of color at the base of grains. This color at the base of grains is red or yellow as determined by the factor pair for red color, *Rr*.

The seed color of Yellow milo, as the name implies, has been considered yellow in America. According to Rangaswami Ayyangar and associates (*15*), the color of Yellow milo is pink rather than yellow. These authors gave Yellow milo seed color the factorial composition *YYWWRRii*. Since they consider the seed color of Yellow milo not true yellow, probably no varieties grown in the United States would be regarded by them as yellow-seeded. Many of the sorghums imported from India will not mature in this country in field plantings, probably primarily because of length-of-day reaction. Yellow-grained types distinctly different from Yellow milo in appearance have been planted at Chillicothe but have not matured.

Vinall and Cron (*29*), Sieglinger (*24*), and Swanson (*27*) have used the symbol *R* to designate a factor for red seed color. Conner and Karper (*2*) reported seed color inheritance in White milo × Yellow milo, Blackhull kafir × Red kafir, and Blackhull kafir × Pink kafir crosses without assigning symbols. Under the hypothesis of Rangaswami Ayyangar and associates these American studies with segregation of red and white or yellow and white seed colors involve the factors *Ww* for wholeness of color and not factors for red or yellow.

Vinall and Cron (*29*) found a 9 brown-seeded to 7 white-seeded segregation in the F_2 generations of feterita × Blackhull kafir and reciprocal crosses. Both feterita and Blackhull kafir are white-seeded varieties. These authors assumed a factor *B* for brown specks carried by kafir and a factor *S* carried by feterita for a spreader which diffused the brown specks, giving the whole seed a brownish color in F_1; the factors were inherited independently. Sieglinger (*24*) revised the hypothesis and assigned the symbol *B* to brown nucellar layer carried by feterita. The dominant smooth or glossy pericarp of kafir was designated *S*, and with both *B* and *S* present the epidermis was brown. Swanson (*27*) assumed the factor *S* to determine the development of a vestigial mesocarp while the allelomorph, *s*, determined a well-developed starchy mesocarp. The thick mesocarp, *ss*, was said to mask nucellar color and inhibit even slight expression of color in the pericarp from the factor *B*. Regardless of the exact functions of the factor pair *Ss*, it was agreed by these investigators that in the presence of both *B* and *S* the color of seed was buff or brown and *B* and *S* were inherited independently.

The brown washes of Rangaswami Ayyangar et al. (*22*), designated B_1 and B_2, may be assumed to be distinct from nucellar layer, since at the time of their report these workers stated (*p. 88*) that in their experience "the nucellar brown has not been met with," and one of the brown washes was reported to be very closely or absolutely linked with the plant color factors *Qq* (*16*). Later Rangaswami Ayyangar (*14*) reported nucellar layer linked with the blackish-purple plant color gene *q* and therefore linked with brown wash. It may be suggested that his statement (*14, p. 200*) that "almost all the varieties with brown nucellus are (varieties) whose leaf sheaths and glumes are blackish-purple" should be qualified to except broomcorn and the kaoliangs from China, Chosen, and Manchuria. Among the many importations of kaoliangs into America a large majority carry the red plant color and have nucellar layer present.

It may be expected, of course, that with further studies and exchange of materials the different views will be harmonized and the mounting number of distinct factors reported will be recognized by all workers.

DESCRIPTION OF CHARACTERS

PLANT COLOR

The influence of one of the pairs of genes affecting plant color in sorghum is expressed in injured tissue throughout the plant and in mature glumes. In some varieties the injured or decaying seminal and coronary roots of seedlings or older plants, the tissues injured by aphids, chinch bugs, or other insects, particularly in leaf sheaths, the tissues surrounding areas attacked by foliage diseases, and the small spots on seed regularly turn a reddish color; and in these varieties the

mature glumes are some shade of red, usually dark but distinctly of a reddish cast. In other varieties the color is much darker and is definitely blackish. Distinction between the two colors is made without difficulty in good seasons although seasonal conditions and modifiers influence development of color, particularly in glumes.

Vinall and Cron (*29*) reported red glumes dominant to black and a 3:1 segregation in the F_2. Rangaswami Ayyangar et al. (*19*) associated glume color with leaf-sheath color. They designated the dominant factor for red *Q* and the allelomorph for black *q*.[3]

BROWN NUCELLAR LAYER

A brown nucellar layer is present in kernels of some varieties of sorghum and absent in others. This structure is a single layer of thick brown cells (fig. 1). The presence of a nucellar layer can be

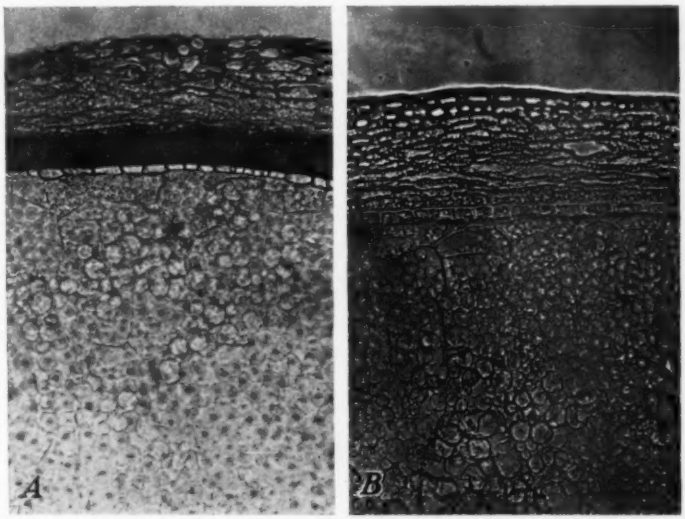

FIGURE 1.—Cross sections of mature sorghum seed showing presence and absence of brown nucellar layer: *A*, Brown hegari (*BBSSrr*); *B*, Dwarf Yellow milo (*bbSSRR*). ×160.

determined readily by scraping off outer layers of the seed coat. Since the symbols *Bb* appear to have been used by Sieglinger (*24*) for brown nucellar layer previous to their use by Rangaswami Ayyangar et al. (*22*) for brown wash, they are used in this paper to represent the factor pair for presence and absence of nucellar layer.

GREEN STRIPED

Chlorophyll-deficient types occur frequently in sorghum. This particular chlorophyll deficiency was observed in a plot of (Blackhull kafir×feterita)×Blackhull kafir, F. C.[4] 8951, at Chillicothe in 1932.

[3] In some American varieties the red color of plant and glume is recessive to black, but the interrelationships have not been completely worked out.

[4] F. C. denotes accession number of the Division of Forage Crops and Diseases.

The character was later found to be monofactorial and recessive to normal green. The typical recessive condition can be best described as similar in appearance to the green-striped in corn illustrated by Lindstrom (*9, pl. III*). The dilution of chlorophyll varies from plant to plant and at different stages of growth. Seedlings sometimes emerge yellow but usually are green and turn yellow or striped within 2 or 3 weeks. In some cases no fading of green occurs until plants are 10 or 12 inches tall. Later most of the plants turn green and at maturity cannot be distinguished from normal greens except that frequently short streaks in the leaves fail to develop color. Some plants remain striped to maturity. The character is sometimes lethal, but green-striped seedlings usually survive unless environmental conditions, such as dry weather or chinch-bug infestation, are severe.

Continued selection of extreme types through three generations failed to produce lines that varied from each other or from parent stocks. The character is influenced appreciably by environment and frequently appears on one end of a series of homozygous rows before it can be detected at the other end. In separating phenotypes it has been necessary to stake the recessive plants intermittently over a period of 3 or 4 weeks, but this separation has been reasonably accurate as determined by the F_3 progenies.

LINKAGE OF Q, B, AND Gs

Linkage of glume color and nucellar layer was observed at Chillicothe in 1929 and briefly mentioned in the Texas Station Annual Report of 1930 (*11*). Rangaswami Ayyangar (*14*) has reported the same linkage but has not given the intensity. The linkage between nucellar layer and green-striped was found in 1935. Data accumulated over a period of years for the coupling phase of the two linkages are shown in table 1. Cross-over values of approximately 16 per-

TABLE 1.—*Segregation in the F_2 coupling phase of plant color (Qq) with nucellar layer (Bb) and nucellar layer with green-striped (Gsgs)*

Years	Factors	Total population	Classes				Cross-over	Standard error
			AB	*Ab*	*aB*	*ab*		
							Percent	
1929–36	*Qq* and *Bb*	8,922	6,167	656	649	1,450	16	0.4064
1935–36	*Bb* and *Gsgs*	2,093	1,466	113	121	393	12	.3503

cent between glume color and nucellar layer and 12 percent between nucellar layer and green-striped are indicated.

An F_2 population of Brown kaoliang, S. P. I.[5] 66384 × green-striped F. C. 8951, in which the three characters were brought together in the coupling phase, was grown in 1936. The single-factor inheritance of each character in this particular population is shown in table 2.

The parental combinations and recombinations of the three characters in the F_2 generation are given in table 3. The indicated order of genes and cross-over percentages is *Q* (13.2) *B* (11.3) *Gs*.

[5] S. P. I. denotes accession number of the Division of Plant Exploration and Introduction, formerly Office of Foreign Seed and Plant Introduction.

TABLE 2.—*Classes of plant color (Qq), nucellar layer (Bb), and green-striped (Gsgs) in an F_2 population of Brown kaoliang, S. P. I. 66384, × green-striped ((Blackhull kafir × feterita) × Blackhull kafir), F. C. 8951, grown in 1936, showing single-factor segregation for each of the characters*

Factors	Total population	Classes			χ^2	*P* value between—
		Dominant	Recessive			
		Number	*Number*	*Percent*		
Plant color, *Qq*	1,960	1,470	490	25.00		
Nucellar layer, *Bb*	1,960	1,476	484	24.69	0.097	0.80–0.70
Green-striped, *Gsgs*	1,960	1,484	476	24.29	.533	.50–.30

TABLE 3.—*Parental combinations and recombinations of plant color (Qq), nucellar layer (Bb), and green-striped (Gsgs) in an F_2 population coupling phase of Brown kaoliang, S. P. I. 66384 × green-striped ((Blackhull kafir × feterita) × Blackhull kafir), F. C. 8951, grown in 1936*

Combinations and recombinations	Factors	Population		Percent
		Number	Total	
Parental combinations	*QBGs*	1,246	1,514	77.245
	qbgs	268		
Recombinations between *Qq* and *Bb*	*qBGs*	123	224	11.429
	Qbgs	101		
Recombinations between *Bb* and *Gsgs*	*QBgs*	98	188	9.592
	qbGs	90		
Double recombinations	*QbGs*	25	34	1.735
	qBgs	9		
Total			1,960	
Recombinations and double recombinations.	*Qq–Bb*			11.429+1.735=13.164
	Bb–gsgs			9.592+1.735=11.327
	Qq–Gsgs			
Total				24.491

INDEPENDENT INHERITANCE OF Q B Gs GROUP WITH SOME OTHER REPORTED SINGLE-FACTOR CHARACTERS

The segregation in F_2 of one or more members of the linkage group with certain other characters is shown in table 4. The segregations indicate independent inheritance or cross-over values so high that linkage cannot be detected. The inheritance of each of these characters has been reported as simple 3:1, although a two-factor segregation has also been reported for seedling stem color.

In the counts accumulated at Chillicothe during the past several years, the segregations for some of the characters deviate significantly from a 3:1 ratio. This condition is frequently found in inheritance studies, and various causes may be responsible. In sorghum some phenotypes are hard to classify at times because drought or chinchbug damage retards normal development of characters, and no doubt a few errors in classification have been made. In other cases it is likely that there may have been modifications in gametic ratios or differential germination or emergence of sporophytes. Regardless of the cause of these deficiencies in the recessive classes, there is no reason to assume more than a single-factor difference in any specific case, and

we are here concerned primarily with the relation of these factors with the *Q B Gs* linkage group. Collins' formula (*1*) was used to calculate expected classes for computation of χ^2. In using Fisher's table of χ^2 (*3*) to obtain a *P* value, *n* equals 2 because two entries in the four frequency classes can be made arbitrarily, leaving only 2 degrees of freedom.

TABLE 4.—*Independent segregation in the F_2 generation of miscellaneous characters with members of the linkage group Q (plant color), B (nucellar layer), and Gs (green-striped)*

Independent characters	Factors (segregating pairs)	Population									χ^2	*P* value [1] between—
		Total	Observed F_2				Calculated F_2					
			AB	*Ab*	*aB*	*ab*	*A'B'*	*A'b'*	*a'B'*	*a'b'*		
		Number	Number	Number	Number	Number	Number	Number	Number	Number		
Spreader	*Qq–Ss*	618	384	100	95	39	375	109	104	30	4.438	0.20–0.10
Pericarp color	*Bb–Rr* [2]	3,319	2,485	626	208	------	2,490	622	207	------	.041	.98–.95
	Bb–Rr	6,773	3,943	1,214	1,222	394	3,933	1,224	1,232	384	.448	.80–.70
	Rr–Qq	139	75	30	25	9	76	29	24	10	.189	.95–.90
	Rr–Qq	5,713	3,359	990	1,019	345	3,333	1,016	1,045	319	3.634	.20–.10
Awns	*Qq–Aa*	6,711	3,908	1,317	1,226	359	3,846	1,280	1,189	396	6.034	.05–.02
	Bb–Aa	10,402	5,929	2,024	1,847	602	5,945	2,008	1,831	618	.724	.70–.50
Juiciness	*Dd–Qq*	2,773	1,577	546	485	165	1,579	544	483	167	.042	.98–.95
	Dd–Bb	8,251	4,718	1,470	1,567	496	4,714	1,474	1,571	492	.057	.98–.95
	Dd–Gsgs	1,760	1,008	319	331	102	1,010	317	329	104	.067	.98–.95
Seedling stem color	*Rsrs–Qq*	3,023	1,786	478	572	187	1,766	498	592	167	4.101	.20–.10
	Rsrs–Bb	3,762	2,197	620	694	251	2,165	652	726	219	8.130	.02–.01
	Rsrs–Gsgs	1,148	681	185	214	68	675	191	220	62	.986	.70–.50
Antherless	*Alal–Bb*	428	241	97	71	19	246	92	66	24	1.795	.50–.30
	Alal–Gsgs	133	75	22	28	8	75	22	28	8	------	------
Waxy	*Qq–Wxwx*	1,971	1,232	322	347	70	1,245	309	334	83	3.225	.20–.10
	Bb–Wxwx	1,971	1,224	309	355	83	1,228	305	351	87	.295	.90–.80
Virescent yellow	V_3v_3–*Bb*	1,029	630	185	161	53	626	189	165	49	.535	.80–.70
Twin-seeded	*Bb–Tsts*	188	100	37	35	16	98	39	37	14	.538	.80–.70

[1] *n*=2.
[2] Ratio expected, 12:3:1.

Pericarp color—this term being used to exclude the brown resulting from the presence of both *B* and *S* (*27*)—was found to be independent of *B* by Vinall and Cron (*29*), Sieglinger (*24*, *25*), and Swanson (*27*). In table 4, independent segregation of *Bb* and *Rr* is shown in two groups of data. The first group contains the factors *SS*, and all phenotypes having nucellar layer present (*B*) are brown. A 12 brown : 3 red : 1 white segregation is expected. In the second group phenotypes *R* and *r* were determined from stigma color, and the expected segregation is 9 *BR* : 3 *Br* : 3 *bR* : 1 *br*.

Likewise two groups of data are given with *Rr* and *Qq* segregating. The first group shows counts among the homozygous *ss* plants of a larger homozygous *BB* population with 9 *QR* : 3 *Qr* : 3 *qR* : 1 *qr* expected. In the second group *R* and *r* were determined from stigma color. The *R* factor appears to segregate independently with both *B* and *Q*.

As previously mentioned, on the hypothesis of Rangaswami Ayyangar et al. (*20*) these segregations involve the *Ww* (wholeness of color) factors rather than the *Rr* factors. The separations at Chillicothe were made on the basis of presence or absence of color on exposed portions of the grain or in stigmas, and no critical study of seed color factors has been made.

The segregation of awnless and awned lemmas apparently is independent of *Qq* and *Bb*. The *P* value for the observed segregation of *Qq* with *Aa* is low and on the border line of significant deviation from the expected, but the segregation of *Bb* with *Aa* is well within expectation, and there is no indication of linkage. Sieglinger (*25*) found no association in inheritance of awns and nucellar layer.

The symbols *Rr*, designated by Karper and Conner (*6*) to represent red seedling stem and the recessive allelomorph, green seedling stem, were used in several earlier papers to represent the factors for red and white seed. It is suggested, therefore, that *Rsrs* be used as symbols for red and green seedling stems. In the counts recorded in table 4 the segregation of *Rsrs* with *Bb* deviates significantly from the expected for independent inheritance. On the other hand, no significant deviation occurs in the segregation of *Rsrs* with either *Qq* or *Gsgs*, and in the chromosome group the latter two pairs are located on opposite sides from *Bb*. Considering all of the data, there seems to be no indication of linkage between *Rsrs* and the *Q B Gs* group.

Segregation of the *Qq* and *Bb* factor pairs with starchy and waxy endosperm indicates independent inheritance. Tabulations of glume color with endosperm and nucellar layer with endosperm were made from the same F_2 populations. *Wx* and *wx* were determined from pollen tests.

The F_2 population was small, but the available data indicate independent inheritance of the factors for twin-seeded spikelets and nucellar layer. Since no symbols for the factors for twin-seeded and single-seeded spikelets have been given previously, *Ts* and *ts*, respectively, are suggested.

SUMMARY

Other workers have reported three linkages in sorghum in which cross-over percentages have been determined. The phenotypes, factor symbols, and cross-over percentages are as follows: Red or green seedling stems (*Rsrs*) and green or albino seedlings (*Ww*), 41.34 percent; starchy or waxy endosperm (*Wxwx*) and green or lethal yellow seedlings (Y_2y_2), 26.5 percent; and purple or brown leaf sheaths (*Pp*) and dry or juicy stalks (*Dd*), 30 percent.

This paper reports a sorghum linkage group of three pairs of genes studied in the coupling phase. The contrasting phenotypes are reddish and blackish plant color, conspicuously shown in glumes; presence and absence of nucellar layer; and normal green and green-striped plants. The factor pairs for the first two characters have been designated *Qq* and *Bb*, respectively, by other authors, and symbols *Gsgs* are suggested for the third. Each of these factor pairs shows complete dominance in the F_1 and simple 3:1 segregation in the F_2. The indicated order of genes and cross-over percentages is *Q* (13.2) *B* (11.3) *Gs*.

Independent inheritance, or cross-over values so high that linkage could not be demonstrated, was found for one or more members of the linkage group with each of the following pairs: Presence and absence of spreader (*Ss*); colored and white seed (*Rr*); awnless and awned lemmas (*Aa*); dry and juicy stalks (*Dd*); red and green seedling stems (*Rsrs*); normal and antherless flowers (*Alal*); starchy and waxy endosperms (*Wxwx*); green and virescent yellow seedlings (V_2v_2); and twin-seeded and single-seeded spikelets (*Tsts*).

LITERATURE CITED

(1) COLLINS, G. N.
1924. MEASUREMENT OF LINKAGE VALUES. Jour. Agr. Research 27: 881–891.

(2) CONNER, A. B., and KARPER, R. E.
1923. THE INHERITANCE OF SEED COAT COLOR IN CERTAIN CROSSES IN GRAIN SORGHUM. Jour. Amer. Soc. Agron. 15: 338–344.

(3) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.

(4) GRAHAM, R. J. DOUGLAS.
1916. POLLINATION AND CROSS-FERTILIZATION IN THE JUAR PLANT (ANDROPOGON SORGHUM BROT.). India Dept. Agr. Mem., Bot. Ser. 8: [201]–216, illus.

(5) KARPER, R. E.
1933. INHERITANCE OF WAXY ENDOSPERM IN SORGHUM. Jour. Heredity 24: 257–262, illus.

(6) ——— and CONNER, A. B.
1931. INHERITANCE OF CHLOROPHYLL CHARACTERS IN SORGHUM. Genetics 16: 291–308.

(7) ——— QUINBY, J. R., and JONES, D. L.
1934. INHERITANCE AND IMPROVEMENT IN SORGHUM. Tex. Agr. Expt. Sta. Ann. Rept. 47: 65–66.

(8) ——— and STEPHENS, J. C.
1936. FLORAL ABNORMALITIES IN SORGHUM. Jour. Heredity 27: 183–194, illus.

(9) LINDSTROM, E. W.
1918. CHLOROPHYLL INHERITANCE IN MAIZE. N. Y. (Cornell) Agr. Expt. Sta., Mem. 13, 68 pp., illus.

(10) MARTIN, JOHN H.
1936. SORGHUM IMPROVEMENT. U. S. Dept. Agr. Yearbook 1936: 523–560, illus.

(11) QUINBY, J. R.
1930. SORGHUM GENETICS. Tex. Agr. Expt. Sta. Ann. Rept. 43: 135.

(12) RAMANATHAN, V.
1924. SOME OBSERVATIONS ON MENDELIAN CHARACTERS IN SORGHUM. Jour. Madras Agr. Students' Union 12: 1–17, illus.

(13) RANGASWAMI AYYANGAR, G. N.
1935. JUICINESS AND SWEETNESS IN SORGHUM STALKS. Madras Agr. Jour. 23: 350–352.

(14) ———
1936. LINKAGE BETWEEN THE BLACKISH PURPLE OF SHEATH AND GLUME, AND NUCELLAR BROWN IN SORGHUM. Current Sci. 5: 200–201.

(15) ——— PANDURANGA RAO, V., and KUNHIKORAN NAMBIAR, A.
1935. THE INHERITANCE OF SOME CHARACTERS IN CROSSES WITH THE SORGHUMS, MILO AND KAFIR. Indian Acad. Sci. Proc. 2: 508–522, illus.

(16) ——— SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1934. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. V. LINKAGE BETWEEN SHEATH, GLUME AND DRY ANTHER, GRAIN COLOURS. Indian Jour. Agr. Sci. 4: 90–95, illus.

(17) ——— SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1937. LINKAGE BETWEEN PURPLE LEAF-SHEATH COLOUR AND JUICINESS OF STALK IN SORGHUM. Indian Acad. Sci. Proc. 5 (B): 1–3, illus.

(18) ——— SANKARA AYYAR, M. A., PANDURANGA RAO, V., and KUNHIKORAN NAMBIAR, A.
1936. MENDELIAN SEGREGATIONS FOR JUICINESS AND SWEETNESS IN SORGHUM STALKS. Madras Agr. Jour. 24: 247–248.

(19) ——— VIJIARAGHAVAN, C., GOMATHINAYAGAM PILLAI, V., and SANKARA AYYAR, M. A.
1933. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. II. PURPLE PIGMENTATION ON LEAF-SHEATH AND GLUME. Indian Jour. Agr. Sci. 3: 589–594, illus.

(20) RANGASWAMI AYYANGAR, G. N., VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1933. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. III. GRAIN COLOURS: RED, YELLOW, AND WHITE. Indian Jour. Agr. Sci. 3: 594–604, illus.

(21) ——— VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1933. STUDIES IN SORGHUM—THE GREAT MILLET. II. ANTHER, STIGMA AND GRAIN COLOUR AFFINITIES. Indian Jour. Agr. Sci. 3: 605–608, illus.

(22) ——— VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., PANDURANGA RAO, V., and SUBRAMANYAM, P.
1934. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. IV. BROWN GRAINS. Indian Jour. Agr. Sci. 4: 81–89.

(23) REED, GEORGE M.
1930. A NEW METHOD OF PRODUCTION AND DETECTING SORGHUM HYBRIDS. Jour. Heredity 21: 133–144, illus.

(24) SIEGLINGER, JOHN B.
1924. SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES. Jour. Agr. Research 27: 53–64.

(25) ———
1933. INHERITANCE OF SEED COLOR IN CROSSES OF BROWN-SEEDED AND WHITE-SEEDED SORGHUMS. Jour. Agr. Research 47: 663–667.

(26) ——— SWANSON, A. F., and MARTIN, J. H.
1934. INHERITANCE OF AWN DEVELOPMENT IN SORGHUMS. Jour. Agr. Research 49: 663–668, illus.

(27) SWANSON, ARTHUR F.
1928. SEED-COAT STRUCTURE AND INHERITANCE OF SEED COLOR IN SORGHUMS. Jour. Agr. Research 37: 577–588, illus.

(28) ——— and PARKER, JOHN H.
1931. INHERITANCE OF SMUT RESISTANCE AND JUICINESS OF STALK IN THE SORGHUM CROSS, RED AMBER × FETERITA. Jour. Heredity 22: 51–56, illus.

(29) VINALL, H. N., and CRON, A. B.
1921. IMPROVEMENT OF SORGHUMS BY HYBRIDIZATION. Jour. Heredity 12: 435–443, illus.

(30) WOODWORTH, C. M.
1936. INHERITANCE OF SEEDLING STEM COLOR IN A BROOMCORN-SORGHUM CROSS. Jour. Amer. Soc. Agron. 28: 325–327.

FURTHER INVESTIGATIONS OF BROWN-STAINING FUNGI ASSOCIATED WITH ENGRAVER BEETLES (SCOLYTUS) IN WHITE FIR [1]

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INTRODUCTION

In a previous paper (*13*)[3] the writer reported an apparently constant occurrence of a pronounced brown stain associated with egg galleries of *Scolytus ventralis* Lec. in white fir (*Abies concolor* Lindl. and Gord.). This stain was found to be caused by a fungus identified as *Trichosporium symbioticum* Wright.

A somewhat similar stain was later observed associated with the egg galleries of two other species of engraver beetles, *Scolytus praeceps* Lec. and *S. subscaber* Lec. This stain was a lighter brown and was usually less evident than that caused by *Trichosporium symbioticum*. Both stains are shallow and are confined to the current annual ring. The studies reported here were made to determine the cause of the lighter coloration, i. e., whether it is due to somewhat different environmental conditions occasioned by location or to another fungus.

These investigations were confined to white fir in California, although red fir (*Abies magnifica* Murr.) and other western evergreens are also known to be infested with these engraver beetles (*11*).

HABITS OF THE BEETLES

A brief description of the habits of the different beetles appears desirable for a better understanding of the fungus associations. More complete information can be found in a recent publication by Struble (*11*).

The galleries of the three different species of *Scolytus* vary in size, extent, and design, and can therefore be readily distinguished. The egg galleries characteristically groove the inner bark and adjacent sapwood. *Scolytus ventralis* egg galleries are confined to the cambial region of white fir trunks and generally occur where the diameter of the tree is more than 4 inches. It is not uncommon, however, for the tops of these same trees to be attacked by *Scolytus praeceps*. The egg galleries of the latter species are likewise found in the cambial

[1] Received for publication June 6, 1938; issued December 1938.

[2] Parts of the studies reported herein were carried on in direct cooperation wirh the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and it is a pleasure to acknowledge the assistance of members of that Division, particularly George R. Struble. Grateful acknowledgment is also made to Dr. Lee Bonar, of the botany department, University of California, for his aid in determining the identity of the staining fungus.

[3] Italic numbers in parentheses refer to Literature Cited, p. 772.

region, but usually where the diameter of the tree is less than 4 inches (fig. 1). Large limbs and sapling white firs are also infested by this beetle. The third species, *S. subscaber*, prefers gnarled and mistletoe-infected branches in which to establish broods. The larvae of all three species characteristically feed in the stained areas surrounding the egg galleries. *S. ventralis* is considered of major importance and frequently causes the death of true firs. Infestations of *S. praeceps* and *S. subscaber*, particularly the latter, appear somewhat less important.

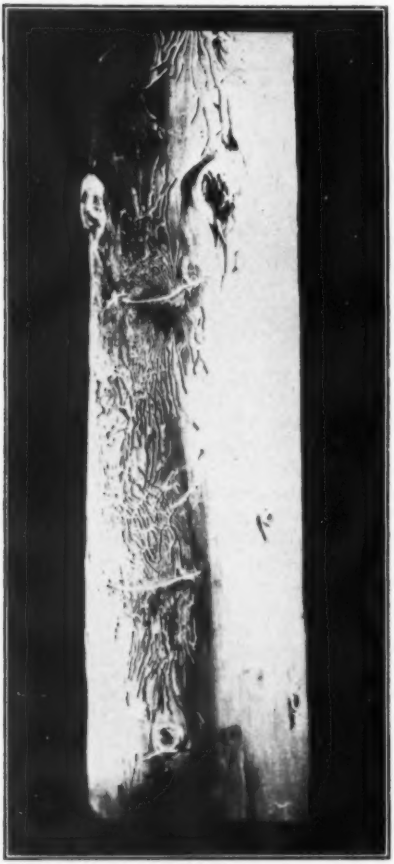

FIGURE 1.—Top section of an infested white fir tree with bark removed to show the stain associated with *Scolytus praeceps* galleries. (Approximately one-third actual size.)

ISOLATION RESULTS

Isolations were secured from stained wood and bark surrounding attacks of *Scolytus praeceps* and *S. subscaber by* cutting away surface contamination with a flamed scalpel. Individual pieces were then removed aseptically and placed on malt agar contained in Petri dishes. Transfers were made later into test-tube slants. Table 1 presents the results of these isolations.

The data presented indicate a fairly consistent fungus association. *Trichosporium symbioticum* was obtained in these isolations only when *Scolytus ventralis* galleries were present in areas adjacent to *S. praeceps* broods at points where the diameter of the tree approached 4 inches. Here the galleries of the two species of beetles sometimes intermingle. These brown stains apparently occur commonly only in association with the respective *Scolytus* beetle galleries.

Fructification of the *Spicaria* staining fungus occurs regularly within the beetle galleries in the form of small, whitish masses of spores. These spore aggregations can be readily mistaken for fresh *Scolytus* beetle frass. The fungus isolated appears entirely different in culture from *Trichosporium symbioticum*, macroscopically as well as microscopically.

TABLE 1.—*Summary of isolations from Scolytus praeceps and S. subscaber attacks in white fir over a 3-year period*

Species of beetle and part of tree attacked	Location of trees	Trees	Beetle attacks from which cultures were made	Isolations showing—		
				Specific fungus	Other fungi	No organism
Scolytus praeceps:		*Number*	*Number*	*Number*	*Number*	*Number*
Inner bark and xylem.	Stanislaus National Forest	4	56	50	4	2
Do	Yosemite National Park	1	12	10	1	1
Inner bark	Sierra National Forest	1	8	8	0	0
Total		6	76	68	5	3
Scolytus subscaber:						
Xylem	Tahoe National Forest	1	12	9	1	2
Inner bark	Sierra National Forest	1	6	4	2	0
Total		2	18	13	3	2

IDENTITY OF STAINING FUNGUS AS SPICARIA ANOMALA

TAXONOMY

The staining fungus isolated has been classified as belonging to the Verticilliae. The writer considers it to be *Spicaria anomala* (Corda) Harz. In 1838 Corda (*2, v. 2, p. 18, table 11, fig. 75*) named the fungus *Penicillium anomalum* and illustrated it. He originally found the organism on splinters of coniferous wood near Prague. Harting (*4*) later created the genus *Spicaria* (1846). In 1871 Harz (*5*) transferred several of Corda's species of *Penicillium* to the new genus and was the first to comment on the characteristic divergent spore chains. The fungus isolated in the present study does not appear to be the more commonly reported species, *Spicaria elegans* (Corda) Harz, since the upright conidiophores are shorter and contain fewer septa. So far as the writer is aware, this appears to be the first published report on the occurrence of *S. anomala* in association with *Scolytus praeceps* and *S. subscaber* egg galleries.

In designating this fungus *Spicaria anomala*, the writer desires to emphasize the fact that considerable variation was found in the number of main branches that extend from the conidiophore stalks. In this respect the isolates from white fir appear atypical when compared with Corda's illustrations. The variations are not considered sufficient, however, to warrant the establishment of a new species or even a strain.

MORPHOLOGICAL CHARACTERISTICS

Spicaria anomala has already been adequately described, and only the important characteristics of the species will be given here as applying to isolates from white fir. Under the low power of a binocular microscope (20 ×), the upright stalks with the accompanying chains of spores resemble miniature, white, bushy trees. Under higher power (40 ×) the branches are seen to occur verticillately in threes around the conidiophore stalks with ultimate ramuli also generally in threes. The spores are borne in great profusion as single chains

from the apex of each ramulus. Individual spores are hyaline, ovate, and 5μ to 6μ in diameter. In potato-dextrose agar (pH 5.5) the submerged hyphae of *S. anomala* are plainly septate, and in old cultures (5 months) attain a maximum width of 5.5μ, averaging approximately 3.0μ. Aerial conidiophore stalks average about 3.5μ in diameter, with branches occurring at a height of 20μ to 40μ. The stalks are usually not more than five-septate. The total height of the conidiophores with chains attached is approximately 150μ (fig. 2).

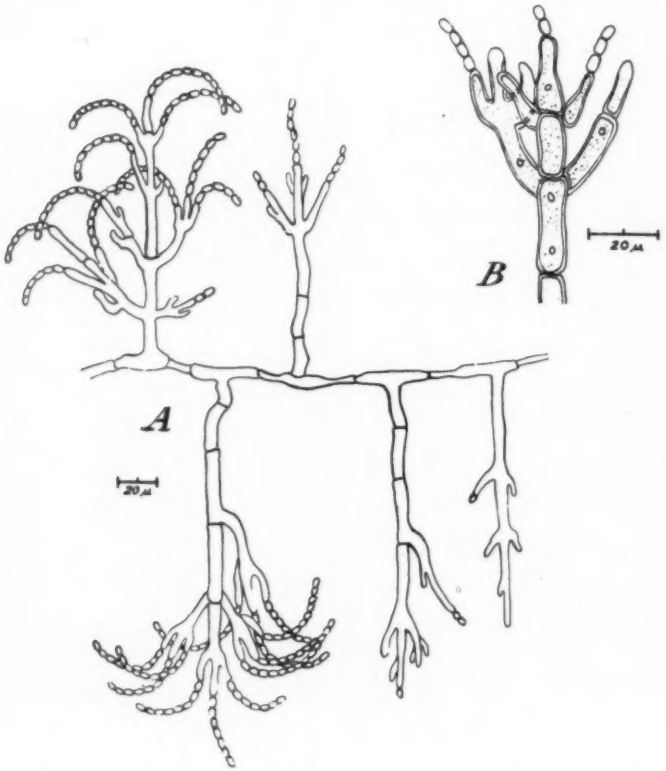

FIGURE 2.—Diagrammatic drawings of *Spicaria anomala* (Corda) Harz grown on potato-dextrose agar (pH 5.5). *A*, Mature and immature aerial conidiophores showing typical branching, septations, and ramuli formations with catenulate spores. Normally the branches are whorled verticillately around the conidiophore stalks. *B*, Details of conidiophore, illustrating the manner of spore attachment to the apex of each ramulus.

Spicaria anomala formed white to cream-colored, zonate colonies and readily produced conidiophores and chains of conidia on all the media on which it was grown (malt, potato-dextrose, corn-meal, and Czapek's agars) (fig. 3). A brown coloration was imparted to the

medium, particularly to potato-dextrose agar, increasing in intensity with the age of the culture. Since the hyphae remained hyaline, the coloration of the agar probably was due to the break-down of certain nutrients. Coloration was less intense in the other agars used. It may be significant that *Trichosporium symbioticum* hyphae were colored brown most readily in malt agar and less readily in potato-dextrose agar.

Microscopic examinations of sections of infected wood stained by the Cartwright method (*1*) showed the hyphae of *Spicaria anomala* in the tracheids and medullary ray cells. No conidiophore formation was seen, however, within the tracheids as reported for *Trichosporium symbioticum*. The hyphae were very delicate as compared with those of other staining fungi, but were well distributed throughout the

FIGURE 3.—A 14-day-old culture of *Spicaria anomala* growing at 22° C. on potato-dextrose agar, illustrating typical zonations. Three-fourths actual size.

stained areas. They regularly entered the medullary rays and tracheids through the pits. They did not appear, however, to concentrate in the ray parenchyma to the same extent as do the blue-staining fungi (*Ceratostomella* spp.). No method of differential staining that clearly showed the presence of the fungus hyphae in the phloem was found; therefore isolations proved the only reliable evidence of their presence in these tissues.

SCOLYTUS BEETLES AS CARRIERS OF STAINING FUNGI

To determine to what extent *Scolytus praeceps* adults carry *Spicaria anomala*, a number of these beetles were collected as they emerged from infested logs contained in entomological cages. They were dropped individually into separate Petri dishes containing lukewarm

agar, where they swam about vigorously until the agar solidified. Later the beetles were removed with sterile forceps, and the plates were incubated. The fungus colonies secured were identified microscopically, and it was found that 66 out of 75 plates contained the specific fungus *S. anomala*. In six of the plates an overgrowth of molds retarded the growth of other fungi, while three of the plates remained sterile. Figure 4 shows colonies of *S. anomala* secured

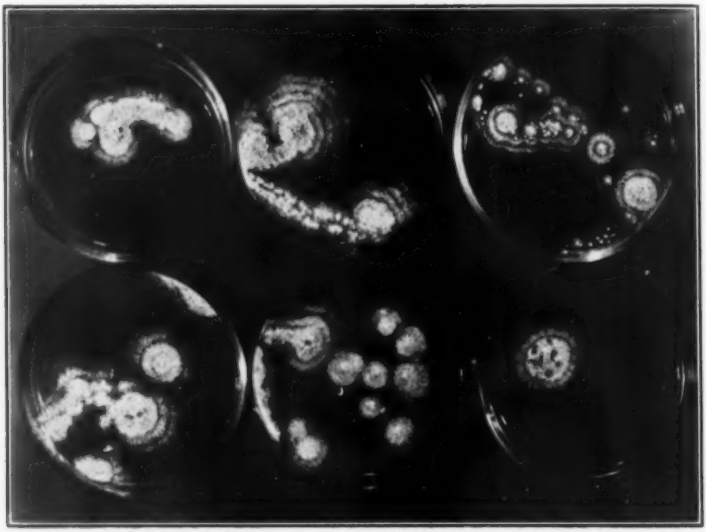

FIGURE 4.—Six Petri dish cultures secured from individual *Scolytus praeceps* beetles at time of emergence. The beetles were dropped into lukewarm agar, where they swam about vigorously until it solidified.

directly from *Scolytus praeceps* beetles in the manner described above. Similar tests were not made with *S. subscaber*, since no adults could be obtained.

The above-mentioned cultural isolations indicate that adult *Scolytus praeceps* beetles carry and regularly introduce *Spicaria anomala* into their egg galleries. Thus, they actually inoculate the cambial region of infested trees, and by the time the larvae begin to feed the stain is well developed. No attempt was made to determine whether the beetles carried the fungus internally or externally. These results, together with the isolations secured from the stained inner bark and xylem, suggest that *S. anomala* is probably the predominant fungus associated with the beetles. Since *Trichosporium* has been previously shown to occur in association with *S. ventralis*, these results, together with certain others obtained outside the present studies, suggest that the relationships are specific and not a matter of chance.[4] Similar conclusions have also been advanced by investigators reporting on other fungi associated with different species of beetles (*7*, *9*, *10*).

[4] In a preliminary investigation of brown-staining fungi associated with the egg galleries of *Scolytus* beetles infesting Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britton), the writer secured a number of isolations of *Trichosporium* sp. from the stained wood surrounding *Scolytus unispinosus* Lec. broods. *Spicaria* sp. was isolated from the tops of the same trees infested with smaller *Scolytus* beetles.

PATHOGENICITY TESTS

A number of inoculation tests were made to determine the pathogenicity of *Spicaria anomala* and to compare the rate of stain spread with that caused by *Trichosporium symbioticum*.

By drilling horizontally between the bark and the wood with a 3/32-inch bit, it was possible to approximate the size and position of the beetle galleries in white fir trunks. The inoculum, consisting of bits of mycelium in agar obtained from pure cultures of *Spicaria anomala*, was inserted into the drill openings. Thus the fungus was placed in direct contact with the cambial region of the trees at diameters ranging from 4 to 9 inches. One hundred inoculations were made in the opposite side of the same standing white firs that had been previously inoculated with *Trichosporium symbioticum*.

All of the inoculations resulted in infection, and at the end of 7 or 8 weeks the average visual longitudinal spread of the *Spicaria* stain in 10 different trees was found to be 3.5 mm per week. This was approximately one-half as fast as the spread of *Trichosporium symbioticum* stain in the same trees. Koch's postulates were followed in a number of cases to prove more definitely that *S. anomala* causes a stain similar to that of *T. symbioticum*. A total of 100 checks made with sterile agar showed no stain development. It was also observed that *S. anomala* killed the cambium as the mycelium advanced, as was concluded for *T. symbioticum*. Both fungi therefore appear to aid the beetles in killing infested trees or parts of trees.

PHYSIOLOGICAL TESTS

Since these studies indicate that the respective staining fungi are associated with certain species of *Scolytus*, it is possible that physiological conditions in the different portions of the tree infested may in some manner favor the establishment of one fungus to the disadvantage of other organisms. Preliminary laboratory and field tests were made in an attempt to analyze some of the possible factors of the environment. The tests were conducted on a comparative basis to determine the differences between the development of *Spicaria anomala* and *Trichosporium symbioticum* under conditions as similar as possible.

EFFECT OF AERATION

An anaerobic apparatus was employed in which pyrogallic acid and potassium hydroxide were used to exhaust the oxygen from a closed container in the usual manner (*3*). Three Petri dishes containing malt agar inoculated with *Spicaria anomala* and three with *Trichosporium symbioticum* were placed on a shelf in this chamber. The covers were not removed from the dishes. After a lapse of 1 week at room temperature (22°–25° C.), radial growth measurements were made.

Under the conditions of these reduced aeration tests, the two species of fungi showed reduction in growth. The growth of *S. anomala* was less adversely affected than that of *T. symbioticum*, but the difference did not appear significant.

EFFECT OF TEMPERATURE

Differences in temperature tolerance might also explain to some extent the restricted occurrence of the stains in the respective parts

of the tree. In the present investigation it was not feasible to make seasonal measurements on the influence of bark thickness and color on the temperature in the cambial region of white fir. However, the effect of temperature on the growth of the respective fungi was compared in the laboratory on the same culture medium.

A series of tests was run in which both organisms were grown on malt agar and subjected to constant temperatures of 5°, 15°, 25°, and 30° C. The results indicate that *Spicaria anomala* is more restricted in temperature range than *Trichosporium symbioticum*. In no case did the growth of *S. anomala* equal that of *T. symbioticum* (fig. 5). Fluctuating temperatures of −15° to +26° C. also favored the growth of *Trichosporium* over that of *Spicaria*.

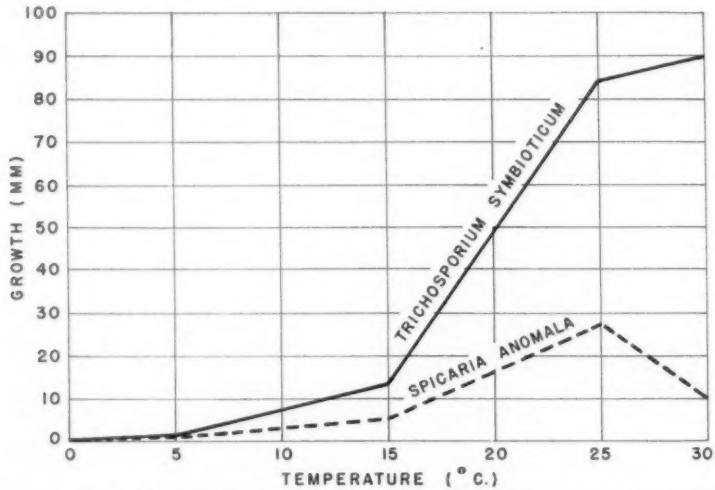

FIGURE 5.—Radial growth of *Trichosporium symbioticum* and *Spicaria anomala* at different temperatures on malt agar for 1 week. (On the basis of measurements secured from triplicate plates subjected to the temperatures of 5°, 15°, 25°, and 30° C.)

The lack of supporting field data makes it difficult to correlate these results with natural conditions. On the basis of these tests, however, the effect of temperature on the growth and development of the respective fungi does not appear to be significant.

EFFECT OF ACIDITY

The development of the respective fungi in solid media adjusted to different degrees of acidity showed that *Trichosporium symbioticum* makes optimum growth at pH 5.0 to 6.0, while *Spicaria anomala* grows best at pH 7.0. In all the acidity tests made, however, the radial growth of *T. symbioticum* always exceeded that of *S. anomala*.

EFFECT OF MOISTURE

Since moisture has been shown to influence the growth of blue-staining fungi by altering the oxygen content of the wood (*8*), it seemed advisable to investigate the comparative growth rates of

Spicaria anomala and *Trichosporium symbioticum* under various moisture conditions. This was done in the field in the following manner:

Five living white firs were partly girdled by chopping a 2-inch-wide band into the wood on one side of the tree, thus stopping normal conduction of moisture on that side. In the same way, five other trees were completely girdled by chopping a similar band entirely around the trunk. Twenty drill inoculations were made in each tree, above the partial or complete girdles, *S. anomala* and *T. symbioticum* being used for 10 inoculations each. The respective inoculations were arranged in two vertical rows on the same side of the trunk. The inoculum, in each case consisting of a small piece of malt agar containing mycelium of the respective fungus, was inserted into the drill holes with sterile needles.

After 2 months the development of the two stains was determined by removing the bark and measuring the average longitudinal spread of each stain above and below the points of inoculation.

To determine the amount of moisture present in the inoculated part of each tree, three samples were extracted from the unstained wood between the two vertical rows of inoculations by means of a 1-inch arch punch.[5] One sample was obtained from the top, another midway, and the third at the bottom of the inoculated regions. In extracting the samples the arch punch was driven through the bark into the wood to a depth of several annual rings. The samples were removed within the circular blade of the arch punch when it was withdrawn. Each sample was immediately wrapped in tin foil and placed in a screw-top container to prevent desiccation before the initial weighing could be made. The bark was removed, and each sample was trimmed at the time of weighing to the thickness of the current annual ring, since, as previously stated, this is the depth of penetration of both stains. The wood cores were then dried at 100° C. until a constant weight was obtained, and the percentage of moisture was calculated on the basis of dry weight. Moisture determinations were made at the time of inoculation and at the end of the tests.

These tests indicated that the maximum visual spread of *Trichosporium symbioticum* stain took place when the current annual ring contained approximately 165 percent moisture, while 155 percent appeared to be the optimum amount of moisture for *Spicaria anomala*. When the moisture was greater than 155 percent, *S. anomala* stain advanced less rapidly in longitudinal spread than did that of *T. symbioticum*.

At the higher moisture contents, however, the brown coloration of *Spicaria anomala* stain was practically equal to that of *Trichosporium symbioticum*. This indicates that the lighter color in association with the *Scolytus praeceps* and *S. subscaber* galleries may be correlated directly with reduced moisture content of the infested parts. When the moisture was reduced to 120 percent, very little evidence of stain could be detected for either species.

The moisture percentages given above are about the same as those reported to favor the development of blue-staining fungi in pine and spruce (*6*). Ordinary blue-staining fungi (*Ceratostomella* spp.) are uncommon, however, in true firs.

[5] An arch punch is a tool used in the leather trade, consisting of a hollow, circular, steel blade with a cutting edge over which a steel handle arches.

EFFECT OF NUTRITION

To study the influence of nutrition on growth, the two fungi were introduced into Petri dishes containing a starvation agar to which various carbohydrates or nitrogen sources had been added. The basic agar was made up as follows:

Magnesium sulphate ($MgSO_4.7H_2O$)	gram	1.0
Monopotassium phosphate (KH_2PO_4)	do	1.0
Calcium sulphate ($CaSO_4.2H_2O$)	do	.5
Agar	do	20.0
Distilled water	liter	1

Eight carbohydrate and seven nitrogen sources were included in these tests. The results obtained from average measurements of the diameter of the colonies in triplicate trials for each compound tested are presented graphically in figure 6.

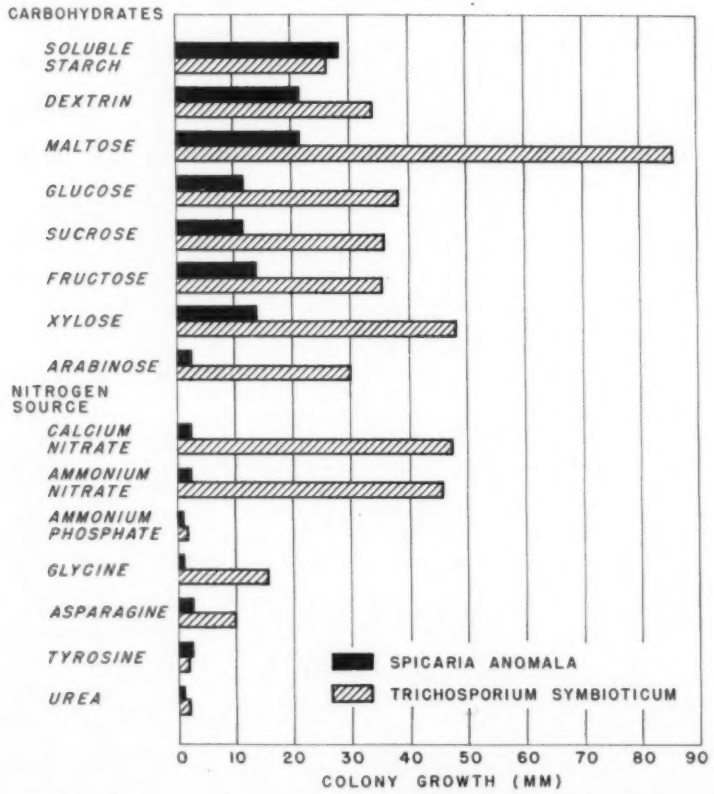

FIGURE 6.—Diagrammatic comparisons of the colony growth of *Spicaria anomala* and *Trichosporium symbioticum* on various nutrient agars at 25° C. on the basis of measurements from triplicate replications over a 10-day period. All media adjusted to pH 6.0.

It may be significant that *Spicaria anomala* made the best growth on the medium containing starch, and the next best growth on the media containing the intermediate hydrolysis sugars, dextrin and maltose. Relatively poor growth was made on the glucose medium. On the other hand, *Trichosporium symbioticum* grew very poorly on starch, made only fair growth on dextrin, and made the best growth on the maltose medium. For both organisms, sporulation was most pronounced in the medium containing the pentose, xylose.

These results indicate that the hydrolysis of polysaccharides is more necessary for good growth of *Trichosporium symbioticum* than of *Spicaria anomala*. Just how significant this might be is not known, since the condition of the trees, seasonal variations, time of infestation, and many other factors may influence nutritional balances. These differences in the utilization of carbohydrates may partially explain how the two fungi can grow and intermingle in the same areas without direct nutritional competition at diameters of about 4 inches where sometimes both *Scolytus ventralis* and *S. praeceps* galleries occur. This might be appropriately termed the region of transition on the trunk, above and below which the respective stains are commonly found alone.

On all the media where there was appreciable growth, with the exception of the starch medium, the development of *Trichosporium symbioticum* has always exceeded that of *Spicaria anomala*.

Spicaria anomala did not appear to utilize nitrogen as readily as did the *Trichosporium*. However, even for the latter fungus the inorganic forms of nitrogen were better. Little growth occurred in the media containing organic nitrogen, but of these the simplest amino acid, glycine, was most favorable. The carbohydrate-nitrogen ratio may also be of importance, although other cultural trials not presented here did not show significant differences in this regard.

On the basis of these tests it appears that physiological conditions probably do not especially favor the establishment of one staining fungus over the other. Instead, it seems most likely that *Spicaria anomala* and *Trichosporium symbioticum* commonly occur in definite regions of the tree only because each fungus is transported there separately by the respective beetles.

INFLUENCE OF THE FUNGI ON THE ENVIRONMENT

It seems possible that *Spicaria anomala* and *Trichosporium symbioticum* may have different influences on certain environmental factors that may favor particularly the respective beetle associations. Several investigators have already indicated independently that wood-staining fungi associated with various beetles may be definitely beneficial, since they create drier environmental conditions in which the larvae develop (*7*, *9*, *13*). To determine the effect of *S. anomala* and *T. symbioticum* on the moisture content of the respective stained regions, a series of tests was made as follows.

Ten white firs 3 to 12 inches in diameter at breast height were selected, and four sets of inoculations were made in each tree. In each set a ¼-inch cork borer (*12*) was employed to remove five bark disks arranged in double bands, one below the other, 2 inches apart.

Three borings, one-half inch apart, were made in the top band with two in the lower band in alternation with those above. Mechanical girdling was thus avoided. Two sets of borings made on opposite sides of the tree were inoculated with *Spicaria anomala* and two with *Trichosporium symbioticum*. The inoculations were made by removing each disk with the cork borer and inserting a bit of agar that contained the mycelium of the respective fungus, after which the disk was immediately replaced. When a band of inoculations was completed, the disks were held in place and desiccation was prevented by covering them with adhesive tape. The inoculum in all cases came from pure cultures.

Two weeks after these inoculations were made samples of the respective stains, one from each set, were extracted between the inoculation bands by means of the arch punch previously described. Duplicate samples of unstained wood were taken from between the inoculation sets. Moisture contents were determined on the basis of the average dry weights of stained and unstained duplicate samples. The green weights of the samples were obtained immediately after extraction to eliminate variations due to air drying. The samples were finally dried at 100° C. until a constant weight was reached. The average weights and the percentages of moisture in the samples are given in table 2.

TABLE 2.—*Moisture content of stained and unstained white fir wood 2 weeks after inoculation (current annual ring only)*

Diameter breast high (inches)	Unstained wood			*Trichosporium*-stained wood			*Spicaria*-stained wood		
	Wet weight	Dry weight	Moisture [1]	Wet weight	Dry weight	Moisture [1]	Wet weight	Dry weight	Moisture [1]
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
3	0.757	0.490	54	0.945	0.607	56	0.922	0.601	53
4	.902	.571	58	.654	.456	43	.735	.522	41
4	.982	.512	92	.601	.398	51	.730	.499	46
5	1.072	.445	141	1.076	.513	109	.833	.496	68
5	1.305	.519	151	.871	.428	104	.873	.468	87
6	1.325	.476	178	1.129	.564	100	1.127	.678	66
9	1.341	.518	159	.857	.468	83	.856	.590	45
10	1.157	.459	152	1.048	.529	98	.819	.524	56
11	1.642	.527	211	.910	.442	106	.926	.515	80
12	1.738	.565	208	1.324	.613	116	1.158	.621	86
Average	1.222	.508	140	.941	.502	86	.898	.551	63

[1] Percentage of moisture based on dry weight.

It should be noted that wood stained by *Trichosporium symbioticum* contained only three-fifths, and the *Spicaria*-stained wood less than half of the moisture found in unstained wood of the same trees. Unstained wood of white firs 4 inches and less in diameter, the size at which *Scolytus praeceps* infestations most commonly occur, had the relatively low average moisture content of 68 percent. Even in these trees the *Spicaria* stain reduced the amount of moisture nearly one-third. The wood of trees greater than 4 inches in diameter, similar in size to those attacked by *Scolytus ventralis*, lost two-fifths of its moisture when stained by the *Trichosporium* fungus associated

with this beetle. In all trees, however, the *Trichosporium* stain dried the wood less than the *Spicaria* stain.

These differences in the amount of moisture present in the wood stained by the two fungi suggested that the respective beetle broods may have different moisture requirements. The larvae of *Scolytus praeceps* appear to require less moisture for development than do those of *S. ventralis*. Each fungus may therefore serve to maintain a favorable moisture balance in infested areas for the broods of associated beetles.

DISCUSSION

The inoculations reported above demonstrate that both *Trichosporium symbioticum* and *Spicaria anomala* can develop in white firs up to 13 inches in diameter. Since neither fungus is apparently restricted by the size of the tree and physiological tests did not reveal any outstanding inhibitions, the inference may be drawn that the respective fungi are confined naturally to separate parts of the tree only because they are introduced there by specific beetles. Isolation results showing that the different species of *Scolytus* adults regularly carry only one staining fungus support this inference.

The association of the stains with respective beetle broods therefore may be of benefit in several ways. *Trichosporium symbioticum* kills the cambium in advance of the development of *Scolytus ventralis* larvae, as was first observed by Struble (*11*). Field observations and cultural results reported here indicate that *Spicaria anomala* in a similar way precedes the galleries of *Scolytus praeceps* and *S. subscaber*. Thus a barrier is provided against the inflow of resin, which might otherwise drown out the broods. This may be of vital importance to the beetles in another respect, since Struble has also observed that white fir resin acts both as a repellent and a toxin to *S. ventralis* adults. Whether the resin has a similar effect on the larvae is not known, but the restriction of resin flow at the time the beetles emerge may be of the utmost importance. The drying of the wood can also influence the environment for the respective beetle brood by correspondingly altering the oxygen content and reducing heat and cold conduction.

It appears from these tests that the presence of the commonly associated fungus that causes the staining of the wood adjoining the egg galleries may well be indispensable to the development of the brood. This has not yet been positively demonstrated by direct experimentation. Likewise, the optimal and limiting factors for brood development and food supply are as yet undetermined. The tests completed, however, suggest a beneficial relationship between the beetles and definite staining fungi.

SUMMARY

Three species of scolytid beetles commonly infest white fir (*Abies concolor*), namely, *Scolytus ventralis*, *S. praeceps*, and *S. subscaber*. The galleries of these beetles are confined to the cambial region, but are characteristically formed in the base, the top, and the branches of the tree respectively.

Brown discolorations have been frequently observed to be regularly associated with the galleries, appearing somewhat less pronounced with those of *S. praeceps* and *S. subscaber*.

Isolations from the stained bark and adjacent sapwood indicate that two different fungi cause the stains. Isolations from newly emerged beetles further suggest definite specificity. The fungus most commonly associated with *Scolytus praeceps* and *S. subscaber* has been identified as *Spicaria anomala*, while *Trichosporium symbioticum* causes a darker discoloration and has been shown previously to be constantly associated with *Scolytus ventralis*.

Inoculations have demonstrated that both *Spicaria anomala* and *T. symbioticum* are pathogenic stains that kill the cambium as they advance. In this way they may aid the beetles in overcoming infested trees.

The respective fungi appear to have a definite influence on the immediate environment. It was found that *S. anomala* reduced the moisture content of the stained wood to a greater extent than did *T. symbioticum*, which in turn reduced the moisture on an average to three-fifths of that of unstained wood.

Physiological studies showed some differences between the two staining fungi, but these do not seem significant. It appears most likely that the respective fungi occur in definite regions of the tree because they are introduced into the egg galleries by the different *Scolytus* beetles.

On the basis of the evidence secured, the association of the brown-staining fungi with the respective beetles may well be of definite aid in the successful establishment and maintenance of individual beetle broods.

LITERATURE CITED

(1) Cartwright, K. St. G.
1929. A satisfactory method of staining fungal mycelium in wood sections. Ann. Bot. [London] 43: 412–413.

(2) Corda, A. C. I.
1838. Icones fungorum hucusque cognitorum. v. 2, 43 pp., illus. Pragæ.

(3) Haldane, J. S.
1920. Methods of air analysis. Ed. 3, 147 pp., illus. London.

(4) Harting, Pieter.
1846. Recherches sur la nature et les causes de la maladie des pommes de terre en 1845. Nieuwe Verhandl K. Nederland. Inst. Wetensch. 12: 203–297, illus. [Original not seen.]

(5) Harz, Carl Otto.
1871. Einige neue hyphomyceten Berlin's und Wien's, nebst beiträgen zur systematik derselben. Bull. Soc. Imp. Nat. Moscow 44(1): 88–147, illus. [Original not seen.]

(6) Lagerberg, T., Lundberg, G., and Melin, E.
1927. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvårdsför. Tidskr. 25: 561–739, illus. [Résumé in Swedish, pp. 692–739.]

(7) Leach, J. G., Orr, L. W., and Christensen, Clyde.
1934. The interrelationships of bark beetle and blue-staining fungi in felled Norway pine timber. Jour. Agr. Research 49: 315–342, illus.

(8) Münch, E.
1908. Die blaufäule des nadelholzes. Naturw. Ztschr. Forst u. Landw. 6: [297]–323, illus.

(9) Nelson, Ralph M.
1934. Effect of bluestain fungi on southern pine attacked by bark beetles. Phytopath. Ztschr. 7: [327]–353, illus.

(10) RUMBOLD, CAROLINE T.
1931. TWO BLUE-STAINING FUNGI ASSOCIATED WITH BARK-BEETLE INFESTATIONS OF PINES. Jour. Agr. Research 43: 847–873, illus.
(11) STRUBLE, G. R.
1937. THE FIR ENGRAVER BEETLE, A SERIOUS ENEMY OF WHITE FIR AND RED FIR. U. S. Dept. Agr. Cir. 419, 16 pp., illus.
(12) WRIGHT, ERNEST.
1933. A CORK-BORER METHOD FOR INOCULATING TREES. (Phytopath. Note.) Phytopathology 23: 487–488.
(13) ———
1935. TRICHOSPORIUM SYMBIOTICUM, N. SP., A WOOD-STAINING FUNGUS ASSOCIATED WITH SCOLYTUS VENTRALIS. Jour. Agr. Research 50: 525–538, illus.

COMPLETE OR PARTIAL INHIBITION OF FLOWERING IN CERTAIN PLANTS WHEN DAYS ARE TOO SHORT OR TOO LONG[1]

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INTRODUCTION

On the basis of their flowering behavior as related to or dependent upon length of day, it has long been recognized that plants may be grouped into three classes—the long-day plants, the short-day plants, and indeterminate plants, i. e., those insensitive to length of day. The long-day plants are induced to flower or flower more quickly as the days are lengthened and the short-day plants as the days are shortened; the indeterminate plants have been so called because their usual flowering appears to be little related to any particular length of day in the natural terrestrial arrangement.

Typical long-day, short-day, and indeterminate plants have been found in abundance. Further studies of the behavior of different plants in relation to length of day have shown that still other plants exist that flower within a definite range of length of day, producing flowers less readily or becoming strictly vegetative when the days are either sufficiently shortened on the one hand or sufficiently lengthened on the other. Since the flowering behavior of these plants in relation to length of day is so distinctive, they have been termed "intermediate" to distinguish them from the long-day, the short-day, and the indeterminate plants.

Relatively few plants showing intermediate flowering behavior have been found. Climbing hempweed (*Mikania scandens* (L.) Willd.) appears to be a typical example of this class, and several other wild plants, including the native wild bean (*Phaseolus polystachyus* (L.) BSP.) and the wild boneset (*Eupatorium torreyanum* Short.), likewise show more or less definite tendencies toward the same behavior. A variety of tropical sugarcane (28 N. G. 292) of the species *Saccharum spontaneum* L. gives evidence of having the sharpest and narrowest flowering range of any plant yet found in this group.

METHODS

In the tests where the entire plant was subjected to the treatment, constant daily light periods of 10, 12, 12½, 13, 13½, 14, 14½, 16, and 18 hours were used. The behavior of the plants in response to the full length of day is taken as the normal seasonal behavior. In those tests with light periods shorter than 16 hours, i. e., from 10 to 14½ hours, inclusive, the plants were kept in large well-ventilated lightproof houses to exclude daylight. The containers with the plants remained upon movable trucks running on tracks and were moved into the daylight on fixed schedules each morning and evening to secure the proper

[1] Received for publication June 20, 1938; issued December 1938.

daylight periods. In all tests involving light periods shorter than the maximum length of the summer day in the latitude of Washington, which is about 15 hours, the light source was natural daylight. However, in those tests requiring 16 and 18 hours of light, respectively, each day, it was necessary to make use of artificial light from sunset. This supplemental light in each test was obtained from four 200-watt incandescent bulbs with reflectors kept at a distance of 1 foot above the plants. In all tests the plants were grown in large galvanized-iron buckets of 14-quart capacity.

EXPERIMENTAL RESULTS

CLIMBING HEMPWEED

Climbing hempweed *(Mikania scandens)* is a perennial usually found in low-ground thickets and is distinctive in being the only species of climbing composite in the Washington region. The slender herbaceous stems die to the ground each autumn, leaving matted rootstocks from which new and vigorous shoots arise each year as soon as spring opens. The flowers also produce seed in abundance, and these tiny propagules, furnished with a hairy pappus, are readily carried away by the winds to establish new plants elsewhere in damp thickets.

Studies were made both with seedlings and with root divisions from a single large rootstock, and the behavior of the plants from these two sources will be presented.

Behavior of Seedlings

Seed of *Mikania scandens* was sown October 5, 1936, in a flat that was kept out of doors until December 7, 1936. The flat was then brought into a cool greenhouse ranging in temperature from 50° to 55° F. Germination took place January 1, 1937, and the small seedlings were pricked off into thumb pots March 3, 1937. Young, vigorous plants having an average height of about 3 inches were transferred to buckets, two in each container, May 18, 1937. On this date the tests in which only natural daylight was used began, while the tests of 16 and 18 hours, requiring supplemental artificial light, began April 22. The flowering behavior for the various constant daily light periods is shown in table 1.

Table 1.—*Flowering behavior of seedlings of Mikania scandens exposed to various constant daily light periods*

Daily light period (hours)	Date of budding	Date of blooming	Height of stem	Remarks
			Inches	
10	June 18	July 9	4	Very few flowers.
12	June 5	June 28	50	Few flowers and flowering soon ceased.
12½	June 10	...do...	50	Many flowers.
13	June 9	...do...	50	Do.
13½	...do...	...do...	50	Do.
14	June 11	June 26	50	Do.
14½	June 8	June 28	50	Do.
16	Aug. 19	Sept. 18	50	1 or 2 flower clusters only.
18 [1]				
Full day	June 22	Aug. 10	50	Many flowers.

[1] Plants failed to bud at this day length.

Behavior of Rootstock Divisions

Divisions from a single rootstock of *Mikania scandens* were potted in 3-inch pots in the cool greenhouse October 26, 1936. Shoots were starting January 21, 1937. These divisions were transferred to buckets, and the tests began May 18, 1937, when the plants were about 30 inches in height. The results are given in table 2.

Table 2.—*Flowering behavior of rootstock divisions of Mikania scandens exposed to various constant daily light periods*

Daily light period (hours)	Date of budding	Date of blooming	Height of stem	Remarks
			Inches	
10	June 30	July 24	50	Four flower clusters only.
12	June 7	July 6	50	Few flowers.
12½	June 11	July 2	50	Do.
13	June 8	June 28	50	Sparse flowering.
13½	June 7	June 25	50	Good flowering.
14	June 12	June 28	50	Do.
14½	June 7	...do...	50	Do.
16 [1]				
18 [1]				
Full day	June 21	July 5	50	Good flowering.

[1] Plants failed to bud at this day length.

The plants derived from rootstock divisions produced very few flowers on lengths of day of 10, 12, and 12½ hours. Flowering soon ceased, and the plants maintained a purely vegetative condition but were less inclined to become scrambling twiners than those that experienced longer light periods.

Localization Tests

Rootstock cuttings of one plant of *Mikania scandens* were made and placed in 3-inch pots October 26, 1936, in the cool greenhouse. These were starting growth January 21, 1937. In April the main stem was cut back to a node within a few inches of the ground in order to stimulate new branches from this node. The plants were transferred to buckets, and the tests began May 29 (fig. 1).

The cases used in these tests were so constructed that a branch conducted inside the case from the plant in the bucket placed just outside was completely excluded from light when the sides of the case were fastened in position. The inside branch was given 10 hours of light each day, i. e., from 6 a. m. to 4 p. m., while an opposite branch from the same node was exposed to the full length of day. Two tests were made. In one the bucket was placed inside the case, with one branch remaining inside and an opposite branch conducted outside; in the other the bucket was placed outside the case, with one branch remaining outside and an opposite branch conducted inside the case.

In the test with the bucket inside the case, the length of the inside branch was 15 inches and that of the outside branch 16 inches, when the test began. The inside branch, given 10 hours of daylight each day, produced buds June 17 and open flowers July 12 at a length of 40 inches. A single flower cluster appeared, and no further flowering took place, the branch otherwise remaining in a vegetative condition all summer. The stem exposed to the full length of day produced buds June 15 and open flowers July 8. This branch was very floriferous throughout the summer.

FIGURE 1.—Climbing hempweed (*Mikania scandens*) in localization tests begun May 29. Portions of the plants inside the case received 10 hours of daylight each day, those outside experienced the full day. *A*, plant with rooted portion outside under the full length of day, and one branch inside. The outside branch showed visible buds June 17 and flowered July 8, the flowers appearing in great abundance. The inside branch never budded visibly. *B*, Plant with rooted portion inside the case, and one branch outside under the full length of day. The inside branch produced one flower cluster only, July 12, then ceased. The outside branch produced buds June 15 and open flowers July 8; very floriferous throughout summer. These plants were grown from divided crowns. Photographed August 13.

In the test with the bucket outside the case, the length of the outside branch was 24 inches and that of the inside branch 13 inches, when the test began. The inside stem, experiencing a 10-hour day, never budded; the outside stem, receiving full daylight, showed buds June 17 and open flowers July 8, at 50 inches.

In both localization tests, the branch receiving only 10 hours of daylight either failed to flower entirely or produced a single cluster of flowers only, showing that this length of day is very unfavorable to flowering.

WILD KIDNEY BEAN

The wild kidney bean (*Phaseolus polystachyus*), a native woodland plant, is the only representative of the genus in the Washington region. It is a scrambling twiner in the woodlands and copses which it frequents, and it flowers in July and August. The stems are herbaceous and die back to the perennial crowns each autumn. This interesting bean is a casual constituent of the flora in some situations of the upper Piedmont, as on the slopes of Bull Run Mountain and Big Cobbler, but its occurrence is usually restricted and only a careful canvass of the woodland undergrowth where it occurs will reveal its presence.

Behavior as Affected by Short Winter Days

The wild bean is very adversely affected by short days of 12½ hours or less. During the autumn of 1936 several hundred plants were grown in the greenhouse from seed that was sown October 4 and germinated October 26. These seedlings were transferred to 6-inch pots November 2, when about 3 inches tall. The young plants soon ceased to grow following the exhaustion of the reserves in the cotyledons.

The foliage on these plants consisted of a pair of simple opposite leaves above the cotyledons, succeeded by a small compound leaf with the usual three leaflets. These leaves were small, thick, crinkled, dark green in color, and never had the appearance of the thin healthy foliage of the summertime. Plants were grown in both cool (50° to 55° F.) and warm (75° to 80°) temperatures, but in neither condition did they show anything but the dwarfed and sickly growth described.

Although the plants are vigorous climbers in response to long summer days, the winter-grown plants gave no hint of elongating stems or twining habit. The plants soon passed into a state of dormancy, followed by loss of foliage, thickening of the stems, and finally death in many instances. It was obvious that the short winter days almost entirely inhibited growth in these plants, which in summertime are accustomed to become much-branched high-climbing vines.

Weak light from a 200-watt mazda bulb with reflector was supplied to a number of these plants from sunset till midnight throughout the winter. The plants quickly responded, becoming leafy twiners, but there was no indication of flowering throughout the winter, perhaps because the light periods, near 16 hours or more in length, were too long. It was evident that a very slight dosage of weak electric light at the end of the natural day sufficed to give the unhealthy winter plants a new lease on life so far as growth and twining were concerned.

Behavior in Response to Different Light Periods in Summertime

Seed of the wild bean were sown March 23, 1937. These germinated April 8 and were transferred to 3-inch pots April 23, and to buckets

May 17, with two plants in each; two buckets were included in each test. At this time the average height of the plants was 2½ to 3 inches. Two series of tests were run, one beginning May 17 and another June 4, but the results were similar. The following results with the different constant light periods were secured in the tests of June 4.

10 hours: None budded, and no twining stems were produced; height 4 inches.

12 hours: One blossom July 12 on one plant; no twining stems; height 4 inches.

12½ hours: One plant flowered July 27 and one August 11; two did not flower; slight twining; height 3 to 4 inches.

13 hours: Three plants flowered sparsely, July 16, July 24, and August 6 respectively; one never flowered; a few short runners produced; height 7 to 16 inches.

13½ hours: Three plants flowered June 23, two July 19, one July 21; runners short, the plants tending to remain bushy; height 22 to 36 inches.

14 hours: Two plants flowered July 8 and one July 27; twining habit strongly developed and plants very floriferous; height 30 to 40 inches.

14½ hours: Two plants flowered July 7 and two July 10; strong twining habit and very floriferous; height 35 to 48 inches.

Full day: Plants flowered August 15; strong twining habit and very floriferous; height 40 to 64 inches.

16 hours: Flowered August 16; strong twining habit, but sparse flowering; height 50 inches.

18 hours: Flowered October 1 at 50 inches; strong twining habit, but only a few closed flowers.

Localization Tests

Seed of the wild bean were sown March 23, and germination was evident April 8. The tiny plants were transferred to 3-inch pots April 23, and to buckets June 4. The tests began June 9, the bucket being kept inside the case (fig. 2). These plants were made to produce lateral branches by cutting back the main stem in April. When the tests began, the inside stem, receiving 10 hours of daylight each day, i. e., from 6 a. m. to 4 p. m., was 5 inches in height, and the outside stem, experiencing the full day, was 1 inch in height.

The inside stem remained very short, producing small, dark-green leafage, as in wintertime, and never budded. This portion of the plant was only 6 inches high on August 18.

The outside branch, receiving full daylight, produced buds July 21, and flowers August 2, at 60 inches. The plant was very floriferous until autumn.

BONESET

Boneset (*Eupatorium torreyanum*) is a native perennial composite of the Washington region, the herbaceous stems dying back to the rootstock each autumn. A clump was divided October 1, 1936, and the divisions were transplanted into buckets to remain out of doors throughout the winter. The various tests with these began March 29, 1937. The results were as follows:

10 hours: No buds formed; 14 sterile leafy stems, the tallest 25 inches; stems very lax, tending to bend over.

12 hours: 11 stems, none flowering; height 28 inches; stems weak, lax, arching toward the ground.

12½ hours: 9 stems, one flowering August 4 at 40 inches; stems very lax, arching.

13 hours: 13 stems, all flowering July 7 at 30 inches; stems very lax, arching.

13½ hours: Many stems, all flowering July 10 at 36 inches; stems more stiff. upright, but less erect than the controls.

14 hours: Many stems, all flowering July 27 at 35 inches; stems less erect than controls.

14½ hours: Many stems, all flowering August 4 at 33 inches; stems strictly erect.

Full day: Many stems, all flowering August 18 at 38 inches, with broad corymbose heads and erect stems.

FIGURE 2.—Wild kidney bean (*Phaseolus polystachyus*) (*B*) in localization tests begun June 9. Inside portion in bucket received 10 hours of daylight each day, i. e., from 6 a. m. to 4 p. m. The branch inside grew but little beyond the original height, 5 inches, at the time the tests began, and finally died in late summer. The branch conducted outside, which received the full day, grew with great vigor, buds being visible on July 21 and open flowers on August 2. This branch was very floriferous, as shown by the pods developed. Photographed August 31. (The plant shown in *A* had no connection with the investigation reported here.)

SUGARCANE

Regulation and timely control of flowering is a matter of great importance in the breeding of sugarcane. Many varieties have shown tendencies to be very irregular in their flowering habits. In order to obtain a better knowledge of the relationship of flowering of some of the sugarcanes to length of day, the Division of Sugar Plant Investigations of the Bureau of Plant Industry arranged tests with a number of their varieties, making use of the large lightproof houses and equipment of the Division of Tobacco and Plant Nutrition.[2] Table 3 shows the growth and flowering behavior of the variety 28 N. G. 292, a form of the species *Saccharum spontaneum* found growing wild in New Guinea by E. W. Brandes, of the Division of Sugar Plant Investigations.

Stalk cuttings of this variety were made in October 1936 and were transferred first to 4-inch and then to 6-inch pots and finally to buckets. The tests were begun about May 1.

TABLE 3.—*Average length and number of stalks produced by the variety of sugarcane 28 N. G. 292 (Saccharum spontaneum) in response to different light periods*

Daily light period (hours)	Average length of stalk	Average stalks	Daily light period (hours)	Average length of stalk	Average stalks	Daily light period (hours)	Average length of stalk	Average stalks
	Inches	*Number*		*Inches*	*Number*		*Inches*	*Number*
10	36.0	14.7	14	45.2	10.6	16	37.5	12.0
12	26.0	13.4	Full day	30.7	8.3	18	41.5	12.0
13	[1] 41.7	12.7						

[1] Flower buds present.

An examination of the stalks revealed the presence of young, developing flowers in response to the 13-hour day only. From the number of stalks shown it would appear that the shorter lengths of day, represented by 10 hours and 12 hours of light each day, favored a more active vegetative condition than the 13- or 14-hour day. It would appear that the additional light from sunset has had little effect upon the behavior of the plants.

It is evident that this sugarcane has shown a very striking behavior, which places it in the intermediate class. This behavior is especially interesting since it shows an exceedingly narrow range of flowering, lying somewhere between 12 and 14 hours.

DISCUSSION

It is obvious that *Mikania scandens* finds conditions most favorable for flowering when the days are neither too short nor too long, or within a range of 12 or 12½ hours to 15 or 16 hours (figs. 3 and 4).

On the basis of its length-of-day behavior, the prediction could have been made that the northern limits of its range would be found somewhere within the latitudes of New England. As a matter of fact its range actually extends from southern Maine to Florida and Texas. It is probable that interactions between lowered temperatures, short summers, and the unfavorable influence of very long days help to define the northern limits of distribution of this plant. Length

[2] This work was under the immediate supervision of George B. Sartoris, of the Division of Sugar Plant Investigations, who has very kindly submitted the data for table 3.

of day alone, as it approaches the critical near 16 hours, retards the appearance of flowers about 5 weeks, and it is obvious that further delay can be expected where much cooler and shorter summers prevail.

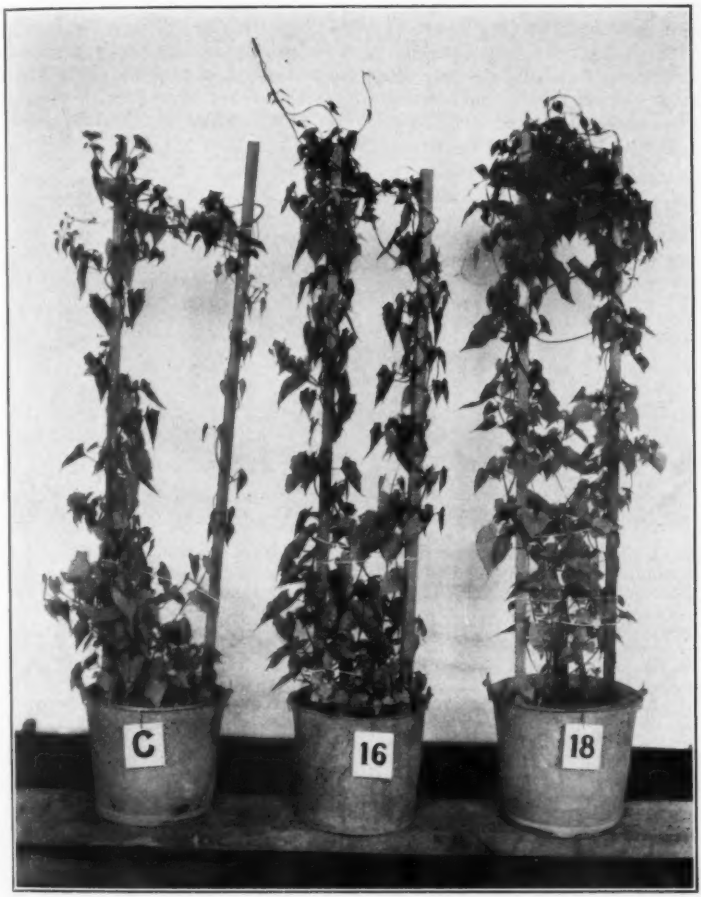

FIGURE 3.—Climbing hempweed (*Mikania scandens*) grown with indicated length of day and with full natural length of day (control, *C*). Plants in *C* flowered August 5, producing flowers in profusion, as is their normal habit. Plants receiving supplemental electric light to afford 16 hours of light produced a few flowers September 18; the plants receiving 18 hours of light never budded.

The summative effects of these factors must make successful flowering and seed production before frost a hazardous event for the species near its northern limits.

On the basis of its length-of-day behavior with respect to the lower limits of flowering, its southern distribution should depend entirely

upon length of day, for unfavorable low temperature is not a factor and the summer season is greatly extended toward the Tropics. All evidence at hand indicates that in its present genetic state the species is not adapted to the shortened days of a tropical habitat, since flowering becomes sparse and uncertain when the days are shortened much below 12 to 12½ hours.

The behavior of the plants grown from seed has been somewhat different from plants derived from old, established crowns. The former did not appear to be affected by the shortened days until a constant length of day between 12½ and 12 hours was reached; the latter when the length of day had fallen somewhere between 13½ and 13 hours.

FIGURE 4.—Climbing hempweed (*Mikania scandens*). General growth behavior of plants with the various periods of natural daylight indicated on the buckets. *C* received the full daylight, serving as a control. Plants receiving 10 hours of daylight daily flowered July 24, producing only four flower clusters. The 12-hour plants, flowering July 6, the 12½-hour plants, flowering July 2, and the 13-hour plants, flowering June 28, all flowered sparsely, indicating unfavorable conditions. Copious flowering was shown when the plants received 13½, 14, and 14½ hours of light each day; flowering taking place June 25 for the 13½-hour plants and June 28 for the 14- and 14½-hour plants. The plants (*C*) receiving the full day flowered July 14, with copious flowering into September. These plants were grown from crown divisions.

The seedlings, likewise, have shown a greater tendency to flower on the higher lengths of day, since there was slight flowering in response to 16 hours of light, whereas the plants developed from rootstocks showed no tendency to flower on the 16-hour light period.

Until a careful analysis of the genetic behavior of the species throughout its range has been determined with respect to its responses to length of day, one cannot, of course, be sure that plants near the northern limits of distribution are identical in their day-length responses with those found in Florida or elsewhere in its range.

The wild kidney bean (*Phaseolus polystachyus*), like *Mikania scandens*, flowers best with an intermediate range of day lengths. Days that are too short or too long are very unfavorable to flowering and may even inhibit it entirely. The normal twining habit of the wild bean is entirely dependent upon length of day. The plants show little or no inclination toward the production of elongate branches or

the twining habit until a length of day between 13½ and 14 hours is experienced, and until the twining habit vigorously asserts itself few flowers are produced.

Vigorous flowering is not strictly correlated with the twining habit, however, since an excessively long day again tends to inhibit flowering, in comparison with the full length of day, as shown by the daily light periods of 16 and 18 hours.

On lengths of day shorter than 12½ hours the plants were of the dwarfed sterile winter type, with rather thick, dark-green rugose leaves and short stems (figs. 5 and 6). They barely survived the

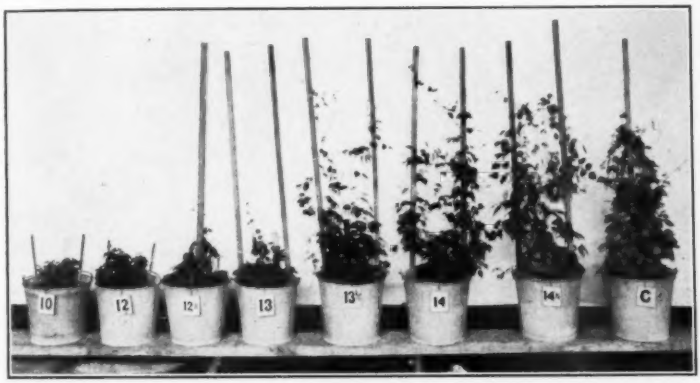

FIGURE 5.—Wild kidney bean (*Phaseolus polystachyus*). General growth behavior of plants under the various periods of daylight indicated on the buckets. *C* represents the full length of day. Plants receiving 10 hours of daylight never flowered. One flower appeared on the plants given 12 hours of light each day. Flowering was more evident with 13 hours of light, but some of the blossoms remained cleistogamic. Plants did not develop the twining habit until 13½ hours of light daily were experienced, but remained low, bushy, and dark green. Copious flowering was the rule with all light periods, including and above 13½ hours. Flowering took place on the 12½-, 13-, and 13½-hour periods from July 24 to July 28; on the 14- and 14½-hour periods, from July 6 to July 10. The controls (*C*) did not flower until August 14.

summer, and were virtually in a state of vegetative dormancy for many months. On those shorter lengths of day near the lower critical length of day for flowering, i. e., 12½ hours, an interesting response asserted itself, characterized by the production of exceptionally large flowers and pods. It is evident that under these extreme conditions leading to an almost complete suppression of twining branches, the few flowers and pods produced were assured a superabundance of available reserve material, since none of it was diverted to the support of rapidly elongating stems.

The natural distribution of the wild kidney bean from Connecticut to Florida is in agreement with its day-length responses and limitations as revealed in the tests. Its inability to flower on the shorter lengths of day and its sparse and delayed flowering when the days are considerably lengthened would preclude a successful invasion into tropical latitudes on the one hand or a far northern distribution on the other, if it is assumed that the genetic constitution is like that which the tests

revealed. The plant probably finds its best adaptation near the middle ground of its range, rather than at the critical extremes where conditions are always more hazardous for any species.

The most striking features of the behavior of *Eupatorium torreyanum* are the development of sterile stems on lengths of day of 12½ hours or

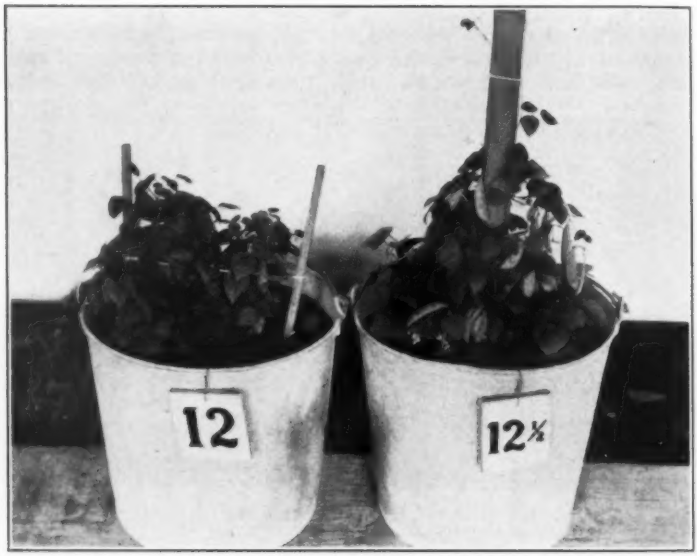

FIGURE 6.—Wild kidney bean (*Phaseolus polystachyus*) grown under lengths of day indicated. Plants receiving 12 hours of light daily never flowered. One of the plants receiving 12½ hours of light flowered July 27; the second never budded. The plants were practically bushy in habit of growth except for one short runner, and produced exceptionally large beans for the species, some of the pods measuring 5.8 cm in length from the tip of the beak to the calyx, with a maximum width of 11 mm. The wild bean makes little growth and can neither twine nor flower when the days are much below 12½ hours in length.

less and the development of very weak, lax, or arching stems until the longest light periods have been reached. The shortest day lengths have strictly inhibited flowering. The 12½-hour day, which appears to be near the critical length for the initiation of flowering as the days are lengthened, greatly delayed flowering as compared with the most favorable day lengths of 13 to 13½ hours (fig. 7). It is obvious that at the higher lengths of day, near 14 hours, flowering is again very noticeably delayed. From the data at hand it would appear that this boneset is not adapted to the extremely long days of northern latitudes nor to the extremely short days of tropical regions, and its natural distribution conforms with these limitations of its day-length behavior, ranging as it does from Pennsylvania to Florida and Texas.

INTERMEDIATE BEHAVIOR AND THE ADAPTATION OF PLANTS

The permanent colonization of plants, whether natural or through human agency, depends upon the inherent adaptive powers of the

species. Since length of day has been shown to be a very potent factor in the control of flowering, it is obvious that the length-of-day responses play a very important part in permanent colonization in any region. The position of the critical length of day for flowering and its sharpness or narrowness may very definitely determine in what latitudes a plant will survive and produce seed before frost. Short-day plants are adapted to distribution in lower latitudes, while long-day plants require the long summer days of higher latitudes. Just how far north short-day plants can succeed depends upon how far into the long-day region the critical length of day is extended, and just how far south long-day plants will spread depends upon how far down toward the shorter days the critical length of day is situated.

FIGURE 7.—Boneset (*Eupatorium torreyanum*) plants grown under the various lengths of day indicated. The control plant (*C*) was grown under the full length of day. Stems were lax and unable to flower on shorter day lengths. A single stem flowered on plant receiving 12½ hours of light; flowering was profuse with 13 hours of light daily, but stems were weak and lax. Stems stiffly erect on control (*C*). The dates of first flowering follow: 12½-hour day, August 4 at a height of 40 inches; 13-hour day, July 7, at a height of 30 inches; full day, August 18, at a height of 42 inches.

Unlike the long-day and the short-day groups, plants of intermediate habit have their limitations determined by two critical points, a maximum in the direction of long days and a minimum in the direction of short days. If the minimum in the direction of the short days is near 13 hours, as in the case of the wild kidney bean, it is evident that the plant cannot flower in latitudes much below 20°, where the longest days of the year scarcely reach this critical length of day.

Eupatorium torreyanum would not flower in the Tropics in latitudes much below 10°, since the minimum critical length of day for this plant is near 12½ hours. On the basis of their length-of-day requirements, *Mikania scandens*, *Phaseolus polystachyus*, and *Eupatorium torreyanum* are not fitted to flower and to reproduce seed near the Equator, where the length of day approaches a constant of about 12 hours throughout the year.

The sugarcane variety 28 N. G. 292 has shown a definite flowering impulse in response to a daily light duration of 13 hours, and a vegetative condition with 12 and 14 hours. It is possible, however, that a length of day approaching 12½ hours and one somewhat longer than 13 hours would also have been favorable to flowering. On this basis this variety can be expected to flower almost on the Equator or at least in tropical latitudes ranging from 4° to 5° up to 20° or more, and this appears to be its normal behavior. This sugarcane has flowered at Guayama, Puerto Rico, practically on the eighteenth parallel, November 9. When this sugarcane is grown in Louisiana near the latitude of 30° N., flowering becomes irregular and may not take place until December. This behavior would indicate that this cane does not find length-of-day conditions very favorable to flowering much outside the Tropics, the northern and southern boundaries of which are 23½° from the Equator. If this behavior is an inherent condition of this variety, as indicated by the very narrow flowering range in the tests, flowering could not be expected in northern latitudes.

In the narrow responses of this cane one sees a highly specialized length-of-day behavior that has not previously been encountered. The very narrow restriction of its flowering responses, however, fits it admirably for naturalization in the Tropics alone, where it has been found at home.

In the case of the sugarcane it must be emphasized that the results presented were secured from a single test. It is thought, however, that the finality of the results of this test indicates the behavior of the variety under the conditions.

The wild kidney bean, *Mikania scandens*, and *Eupatorium torreyanum* are well adapted to middle latitudes lying roughly between 25° and 45°. The north and south range of such intermediate plants depends upon the upper and lower limits of their day-length responses. If these are far apart the range of the plants will be wide; if they are close together the range, as in the case of the sugarcane 28 N. G. 292, will be restricted. In the case of this cane, the capacity to flower lies somewhere within a range in length of day of only 2 hours, and the low upper limit at which flowering fails keeps the plant within the Tropics. While such plants have not yet been found, it is possible that intermediate plants quite as narrow in their length-of-day requirements for flowering will be found to exist in far northern latitudes. Such plants, like those in the long-day class, flowering in response only to very long days, could adapt themselves only to far northern latitudes. On this basis it is possible that some plants require continuous light for their best development.

SUMMARY

A number of plants of intermediate behavior have been found. They constitute a group of plants whose flowering is favored by lengths of day neither too short nor too long. On either side of this optimum range, flowering may cease entirely or be delayed or less profuse.

Mikania scandens, *Phaseolus polystachyus*, *Eupatorium torreyanum*, all native wild plants of Washington, D. C., and the wild New Guinea sugarcane 28 N. G. 292 show the intermediate flowering behavior.

Mikania scandens flowers very poorly in response to lengths of day that are 12 hours long or less, and flowering has practically ceased when the days have been lengthened to 16 hours or more.

Phaseolus polystachyus is near its flowering limit when the days are reduced to 12½ hours, and flowering is again checked or delayed when the days have been lengthened to 16 hours or more.

Eupatorium torreyanum ceases to flower when the length of day has been reduced to about 12½ hours or less. Experiments indicate that the plants flower most readily with 13 to 14 hours of daylight, and show a delay in flowering when the daylight periods exceed these values.

The wild New Guinea sugarcane 28 N. G. 292 of the species *Saccharum spontaneum* L. has shown the narrowest flowering response of any plant yet studied. Tests with daily light periods ranging, at 1-hour intervals, from 10 to 14 hours, and with the full length of day, at Washington, D. C., revealed that flower buds were formed only when the plants were afforded 13 hours of light each day. The flowering range for this species, therefore, lies somewhere between 12 and 14 hours of daylight.

U. S. GOVERNMENT PRINTING OFFICE: 1938